Journal of
DAIRY SCIENCE

Vol. 44 April, 1961 Number 4

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JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
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Advertising plates, copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to the Journal of Dairy Science in care of The Garrard Press, 510-522 North Hickory Street, Champaign, Illinois.

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Membership dues in the Association are $10.00 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgway Circle, White Plains, New York. Renewals should be made not later than December 1 each year.

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PEOPLE AND EVENTS

NEWS FROM THE UNIVERSITIES

California

Dr. G. P. Whitlock to New Position

G. P. WHITLOCK, director of health education for the National Dairy Council, will join the staff of the University of California Agricultural Extension Service on May 1, as program leader in family and consumer sciences

G. P. Whitlock

The 44-yr.-old scientist and teacher will head a growing statewide program to extend knowledge of nutrition and consumer economics to California homes, both rural and urban. He has served both universities and industry, and comes to California as an experienced speaker and director of educational services to medical people, newspaper, radio, and television audiences, and to the general public.

Dr. Whitlock has been head of the National Dairy Council consumer education program since 1956. For 10 yr. before that, he was animal products manager, doing industrial development and technical service work for a large pharmaceutical firm. As a teacher and scientific researcher, Dr. Whitlock has been on the staffs of Pennsylvania State University and Iowa State University. He earned the Ph.D. degree in biochemistry from Pennsylvania State College in 1942.

A native of Illinois, Whitlock took his undergraduate training at Southern Illinois University, where he earned the B.S. degree in chemistry in 1939. He is a member of the American Chemical Society, the Institute of Food Technology, and the American Dairy Science Association.

Illinois

Schaufelberger Wins Scholarship

GEORGE SCHAUFELBERGER, Southern Illinois University agriculture student from Greenville, has been selected by the Southern Illinois Dairy Technology Society for a $100 cash scholarship award to apply to school expenses during the winter and spring terms.

Schaufelberger, a sophomore enrolled in a predairy technology course, is preparing for work in the dairy processing field. He will transfer to the University of Illinois next year to complete his training as a dairy technologist. The Society is composed of dairy industry representatives from the southern one-third of Illinois.

George Schaufelberger, center, sophomore predairy technology student at Southern Illinois University, receives a Southern Illinois Dairy Technology Society cash scholarship grant of $100 from Norman Rushing, Carbondale, Prairie Farms Creamery fieldman representing the Society. At right is Dr. Howard H. Olson, SIU dairy specialist and secretary of the Society.

The student is the son of Mr. and Mrs. George Schaufelberger, Sr., breeders of Brown Swiss dairy cattle.

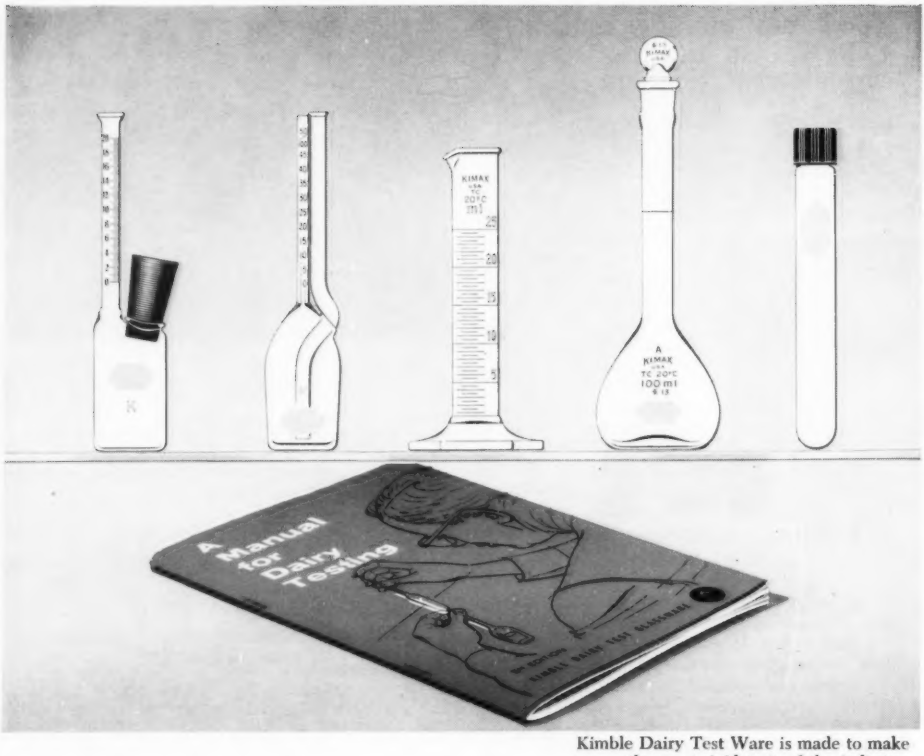
Sixth Annual Dairy Day at Southern Illinois University

Participating in the Sixth Annual Dairy Day on March 2 at Southern Illinois University were: K. E. HARSHBARGER, University of Illinois assistant professor of dairy science; W. E. PETERSEN, University of Minnesota emeritus professor of dairy husbandry; ELMER HANSEN, public relations director of Curtiss Breeding Service, Cary, Ill.; ALEX REED and HOWARD OLSON, chairman and associate professor of animal industries, respectively, Southern Illinois University; and GERALD KRAWCZYK and L. D. STEWART, graduate students in animal industries at SIU.

Dr. Harshbarger discussed feeding practices necessary for producing good replacement heifers for the dairy herd. Dr. Olson spoke on the importance of good replacement heifers. Prof. Petersen emphasized the importance of good milking practices and udder care in modern dairying, where the emphasis is on faster milking and greater production pressure. Mr. Hansen said that breeding, feeding, and farm management must be put together on the farm in such a way that milk production will be higher, at a greater profit to the farmer.

Salisbury Appointed to USDA Panel

G. W. SALISBURY, Head of the Dairy Department, University of Illinois, has been asked to serve on a special panel to consider the research and development program of the U. S. Department of Agriculture. This panel,



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headed by DR. GEORGE HARRER, acting president of Rockefeller Foundation, has been appointed by DR. J. B. WEISNER, special assistant to President Kennedy for Science and Technology.

Harshbarger Appointed to Surgeon General's Staff

K. E. HARSHBARGER has been selected to fill the mobilization and designation position of the Nutrition Branch, Preventative Medicine Division, Office of the Surgeon General, Washington, D. C. This position is the key one in the Army Reserve Program. In the event of mobilization, Dr. Harshbarger would be responsible for organizing and supervising the medical nutrition program.

Dr. E. E. Ormiston to India

E. E. ORMISTON of the University of Illinois Department of Dairy Science, left for India this month to act as advisor in dairy science and dairy science extension at the Benaras Hindu University in Varanase. He will be in India for 2 yr. He will work with researchers and instructors in the Indian University's Department of Dairy Science and with Indian extension representatives.

During Ormiston's absence, J. L. ALBRIGHT will be in charge of dairy farm operations. Dr. W. W. YAPP will handle Dr. Ormiston's teaching duties.

Dr. R. W. Touchberry Awarded Grant

The University of Illinois Department of Dairy Science has received a $45,000 research grant to study the effects of X-radiation on body size, fertility, and longevity of laboratory animals. R. W. TOUCHBERRY will be in charge of the project. The grant was furnished by the National Institutes of Health.

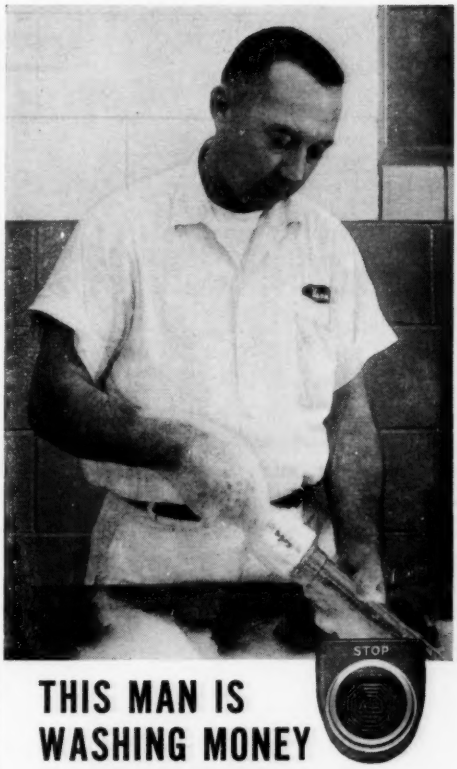
Iowa

G. W. Reinbold Promoted at Iowa State University

G. W. REINBOLD has been promoted to the position of Assistant Professor of Dairy Bacteriology, a position formerly held by F. E. NELSON, who resigned to take a similar professorship at the University of Arizona.

G. W. Reinbold

Dr. Reinbold graduated with the B.S. degree from Pennsylvania State College in 1942. He entered the Armed Forces and was transferred to inactive status in 1946 with the rank of Major, Chemical Warfare Service. In 1947, he earned the M.S. degree in Dairy Technology and in 1949 the Ph.D. degree in



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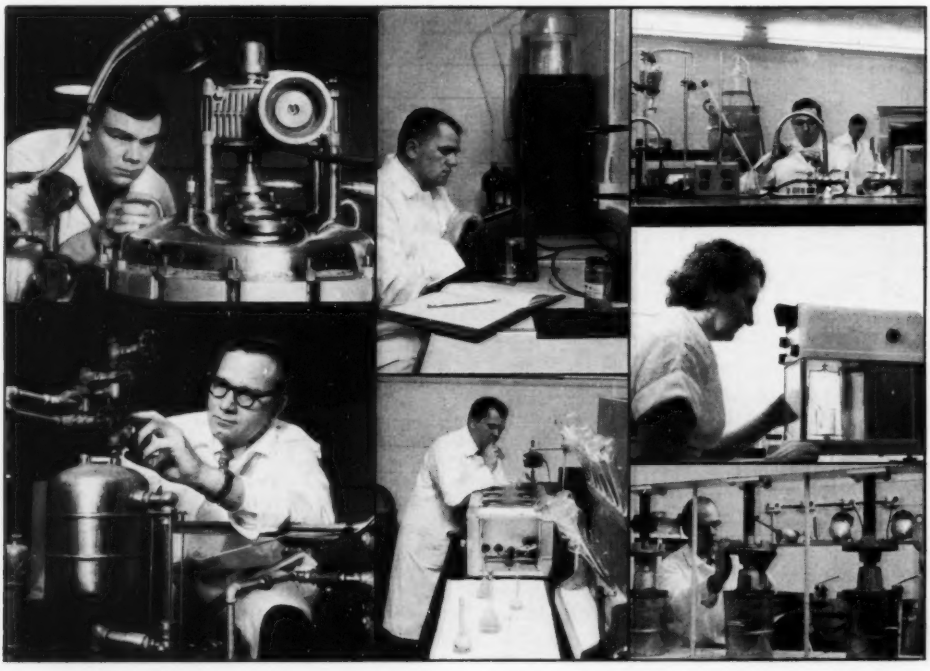

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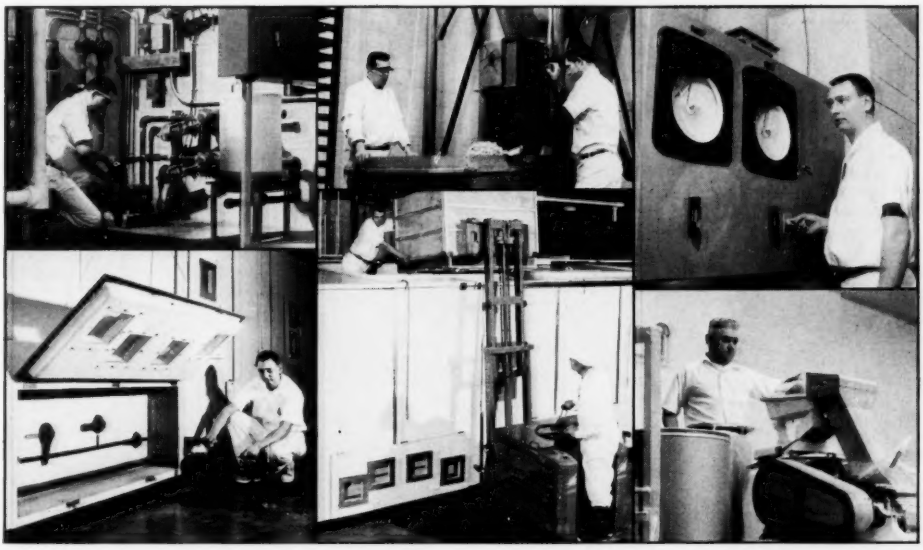

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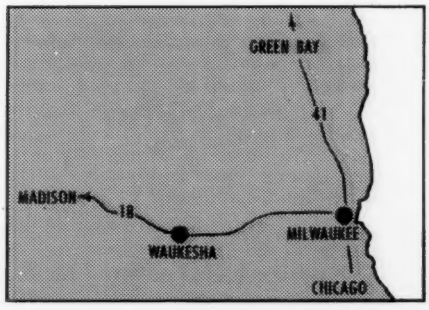

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Dairy Bacteriology, both from the University of Illinois. From 1949 to 1954 he served as research bacteriologist for the National Dairy Products Research and Development Laboratory at Glenview, Illinois. Here he served as leader of the bacteriology group under the direction of R. V. HUSSONG, with whom he did his work for the Ph.D. degree at the University of Illinois. In this position he was awarded the J. L. Kraft Merit Award for information contributing to the bacteriology of cheese. From 1954 to 1958, he was production technician in the General Office of the Kraft Foods Company and was concerned chiefly with the development and improvement of Swiss Cheese.

Dr. Reinbold was Extension Dairyman in Manufactures at Iowa State University from 1959 until he assumed his present duties.

Ohio

Milk Sanitation and Public Health Short Course

The 10th Annual Milk Sanitation and Public Health Short Course was held March 20-24 on the Ohio State University Campus. It was sponsored jointly by the Ohio State University, Department of Dairy Technology, the Ohio Department of Health, and the Ohio Department of Agriculture. Fifty persons participated in the course, which was designed to enable milk sanitarians and dairy plant operators and employees to deal more effectively with problems in milk sanitation. Among the subjects presented were: basic sanitation principles; fundamentals of dairy farm and plant inspection; sanitation features of dairy equipment; dairy product processing; special problems in cleaning and sanitation; sanitation aspects of HTST pasteurization; and interpretation of legal standards and regulations as applied to specific problems. The keynote address of the course was delivered by DR. H. E. CALBERT, University of Wisconsin, who spoke on New Frontiers in Sanitation.

Coordinators of the program were D. D. COLE, Ohio State University, Department of Dairy Technology, R. WATTS and R. MARTIN, Ohio Department of Health, and M. WEIMER, Ohio Department of Agriculture.

R. K. DAVIS has been named Chief of The Food and Dairy Division of the Ohio Department of Agriculture. Davis brings many years of experience in the Department to the position, in which he succeeds E. C. HAAF. Haaf is entering private industry.

O. L. HUNNICUTT has retired after long service to the Ohio Department of Agriculture as Supervisor of the Dairy Section. He is succeeded by H. F. LAWRENCE.

The recruiting program of the Department of Dairy Technology, working with the cooperation of Education Committees of the various Dairy Technology Societies, the Ohio Dairy Products Association, and other Ohio dairy groups, was intensified during April. High lights of the activities were a series of informal open houses held by the Department of Dairy Technology to familiarize prospective students with the facilities of the Department and with career opportunities in the Dairy Industry. On April 29, competitive examinations and interviews were held by the Department to select recipients for the many scholarships made available by the Dairy Industry companies and groups throughout the state. Chairmen of the cooperating education committees of the various groups are: Ohio Dairy Products Association, E. WEBER, Cincinnati; Northeastern Ohio Dairy Technology Society, B. F. FORBES, Cleveland; Maumee Valley Dairy Technology Society, C. J. LUDWIG, Toledo; Cincinnati Dairy Technology Society, W. ZINK, Cincinnati; Central Ohio Dairy Technology Society, JOSEPH PETERS, Marysville, and FRED RUHL, Washington Court House; Columbus Milk Distributors Association, G. LOWER, Columbus; Dairy Foods Foundation of Northeastern Ohio, R. J. HROMCO, Cleveland; Dayton Milk Foundation, V. Z. DORFMEIER, Dayton; Akron Milk Dealers, HAROLD REITER, Akron; and Stark County Dairy Products Association, LEE CAVITT, Canton.

Northeastern Ohio and Maumee Valley societies met on April 5 and 6, respectively. W. J. Harper, Department of Dairy Technology, Ohio State University, who recently returned from a six months' leave of absence during which he visited dairy research centers and processing facilities in Europe, spoke to each group on European dairy practices. The meeting of the Maumee Valley Society was preceded by a tour of the Hawthorn-Mellody Ice Cream Plant at Bowling Green.

Central Ohio. The principal speaker at the April 5 meeting at Columbus was N. J. Crabtree, Education Specialist for the Ohio Division of aviation, who discussed Aviation Today and in the Future and presented a film entitled The Next Ten Years in Space. New officers elected at the meeting were President, Bert Taylor, Columbus; Vice-President, Dean Foster, Columbus; Secretary, F. E. Bright, Waterford; Treasurer, R. Spires, Columbus; Sergeant-at-Arms, Fred Marsh, Columbus. The following new members of the Board of Directors were elected: Gene Lower, Columbus; K. T. Yoder, Columbus; and R. F. Buehler.

Cincinnati. The emphasis at the April 11 meeting was placed on ice cream. Prior to the meeting, B. W. Tharp and D. D. Cole, Department of Dairy Technology, Ohio State University, conducted a clinic on vanilla and chocolate ice cream samples submitted by So-

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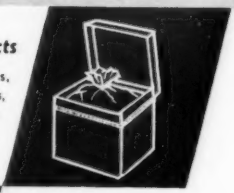

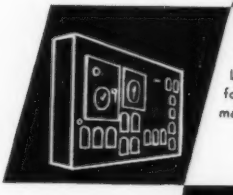

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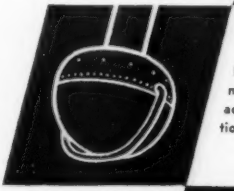

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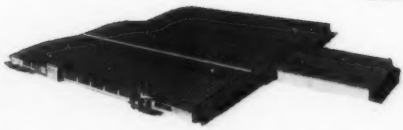
For plant and farm automation; spray-sanitizing; acidified rinsing; proportional feeding.



Doubled Research Facilities

For product development, materials testing, basic research, field testing and trouble-shooting.

ciety members for evaluation. The topic of Dr. Tharp's talk, presented at the dinner meeting of the Society, was Success with Soft Serve.

Minnesota

Dr. Petersen Speaks at Southern Illinois University

W. E. PETERSEN, University of Minnesota emeritus Professor of Dairy Husbandry, lectured to the Southern Illinois University Sigma Xi Club in Carbondale, March 1, 1961. Dr. Petersen's lecture was on Antibodies in Milk for Protection Against Human Disease.

South Carolina

Conference at Clemson College

A conference devoted to the Purpose and Technique of Quality Control was held at Clemson College, S. C., January 24, 25, and 26, 1961. The Dairy Department personnel presented stimulating talks on various phases of quality control, beginning with the farm, through the dairy plant to the consumer. A total of 16 individual topics was discussed, covering such items as: milk sampling procedures, milk fat and bacterial analyses, flavor evaluation and keeping quality, importance of animal health in maintaining top-quality production.

A stimulating panel discussion—Need for and Use of Laboratory Results—was one of the high lights of this conference. Panel members participating were: TOM BURTON, Sales Manager, Sealtest Foods, Columbia, S. C.; C. E. COUSINS, Fieldman, Pet Dairy Products, Co., Inc., Columbia, S. C.; W. M. HOBSON, Manager, Foremost Dairies, Inc., Spartanburg, S. C.; and C. T. SMITH, Production Superintendent, Edisto Farms Dairy, Columbia, S. C.

J. P. LAMASTER, Professor Emeritus of Dairying, Clemson College, provided a very interesting evening program with his Colored Slides and Commentary on the Dairy Industry in Brazil. Prof. LaMaster spent 2 yr. in Brazil as Livestock Advisor, International Cooperation Administration, under the auspices of the U. S. Department of State.

Tennessee

Professor C. E. Wylie Goes to Illinois

C. E. WYLIE left for Southern Illinois University the middle of March with Mrs. Wylie. He will be on the staff of the Illinois Institution for the spring quarter, to help develop a new dairy production course for graduate students. This is made possible by a grant in the Illinois School of Agriculture, to be administered by ALEX REED, Head of the Animal Industries Department.

Professor Emeritus Wylie retired from the University of Tennessee September 1, 1960, after developing the Dairy Department and serving as its first head over a period of 44 yr.

STUDENT CHAPTER NEWS

J. E. JOHNSTON, Editor
A Section Devoted to News of Student Members

Oregon State College Dairy Club Fall Activities Summary

Much the same as the dairy farmer, the gardener, or the housewife, the Oregon State College Dairy Club experienced a busy 1960 fall season. In the evenings, between classes, and sometimes in lieu of classes, the club members rushed about their fall activities.

Since dairy products and dairy cattle judging teams at Oregon State receive no school or departmental help for team members' expenses, the club has organized its treasury to give the greatest possible assistance to both teams.

This year club funds were used to pay hotel expenses for both teams at the Pacific International Livestock Exposition in Portland, Oregon. In addition, the dairy cattle judging team had club funds for travel to the Cow Palace in San Francisco, and the dairy products team with two alternates received sup-

HOW TO MAKE THE WORLD'S BEST ICE CREAM

You must use the finest dairy ingredients, the finest flavors, the finest mix processing and freezing equipment, the finest packaging—*And The Finest Stabilizer*—KELCO STABILIZER.

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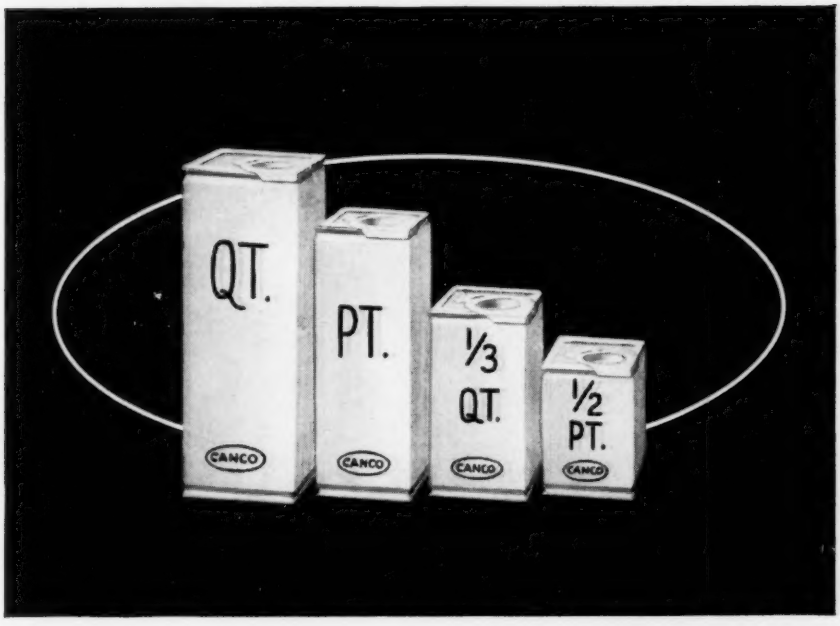

MILK

IN GRADE "A" CARTONS

Canco's paper containers, the original disposable cartons for milk, have been winning greater acceptance every year! Popular with homemakers everywhere, these containers are ideal for use in school cafeterias and vending machines. They are easy to open and close; provide "controlled pouring," and are compact, sturdy, sanitary. Yes, Canco cartons are the *preferred* containers for milk . . . the Grade "A" cartons for nature's most nearly perfect food!

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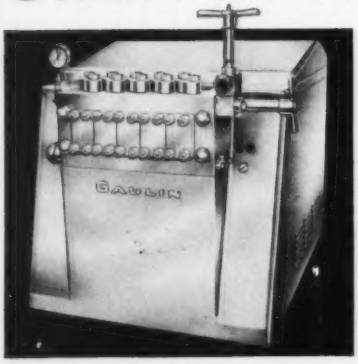
GAULIN ADVANCED TECHNOLOG

Costs Less to START WITH



Take any size Gaulin Homogenizer. Divide its total capacity into its price — and you'll find a Gaulin *costs less per gallon* than any other make on the market.

Of course, you probably won't use the total capacity to begin wit Most dairies want room to grow in.

But the fact is — Gaulins give you *more room* to grow in *at a lower* initial investment.

Add to this Gaulin's lower operating costs, longer life, finer homogenization, and easier cleaning — and you know why more da use Gaulin Homogenizers than all other makes combined.

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port for transportation to the International Dairy Show and Judging Contest in Chicago in November.

To be able to support this type of expensive activities, the club has to have great money-making ability. The dairy club has two primary money-making activities—its coffee, and ice cream drumsticks.

During fall term, club members made some 500 dozen ice cream drumsticks for sale to dormitories and other outlets. With cool, damp Oregon autumn weather, coffee sales were good. The club also served coffee and sweet rolls at the four-day Oregon Agricultural Conference, attended by nearly 400 people.

The OSC Dairy Club holds only one meeting each month unless the executive committee deems it necessary to call a special meeting. Each meeting includes a short program. The program for November 9 was the initiation of seven new members into the club. The initiation of these men, ranging from freshman to graduate, brought the club membership to 28.

At the December 7 meeting, MISS JUDITH GIBSON, Oregon's 1960 Dairy Princess, spoke to the club. She told the group the duties of the Oregon Dairy Princess and what the OSC Dairy Club could do to help her. Miss Gibson was made an honorary member of the club.

No matter how busy, dairy club members always have time for entertainment. This was shown November 10, when the club members participated in Aggie Variety, a night of fun and laughs for all OSC, sponsored by the School of Agriculture.

Michigan State Dairy Club Activities

Members of the Michigan State University Dairy Club visited Green Meadow Farms at Elsie, Michigan, and the Michigan Milk Producers plant at Ovid, Michigan, on their winter-

Michigan State Dairy Club members Lew Meyer, Art Vasold, Warren Suchovsky, Nathan Zimmerman, and Coromoto Perez, discuss milk manufacturing problems with Cy Tremblay, Superintendent of the Michigan Milk Producers plant at Ovid, Michigan.

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It's Just Like Having An Extra Man In Your Quality Control Department

You actually get two-for-one service in dealing with National Pectin. First, you get a personal, highly specialized service to help solve any dairy production or quality control problems. Second, we'll help you select (from the widest possible variety of stabilizers and emulsifiers) the product most suitable to your needs for frozen dairy products or cottage cheese.

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THE INDUSTRY-WIDE SYMBOL OF

POSITIVE MILK PROTECTION

Once again in 1960 Sealright leadership in dairy packaging introduced another Vital First to the Dairy Industry. SEALKING SINGLE-PAK HALF GALLON . . . the first single-unit polyethylene plastic-coated paper half gallon milk carton. It too bears the symbol of POSITIVE MILK PROTECTION. Sealright has long sustained its well deserved reputation for POSITIVE MILK PROTECTION.

Among the major contributions SEALRIGHT has made to the dairy industry are these vital FIRSTS:

1959 . . . Sealright was FIRST with Rim-Print Coverite glass milk bottle capping.

1958 . . . Sealright was FIRST with All Plasti-Cap for glass milk bottle capping!

1957 . . . Sealright was FIRST with introduction of polyethylene Plastic-Coated Sealking!

1956 . . . Sealright was FIRST with Brand Name printing on cover rim of Nestyles!

1954 . . . Sealright was FIRST with Plastic-Coated Nestyle cottage cheese packaging!

1948 . . . Sealright was FIRST with modern Plastic-Coated Sealking paper milk cartons!

1948 . . . Sealright was FIRST with Plastic-Coated Nestyles, quality and flavor protective ice cream packaging!

1938 . . . Sealright was FIRST with revolutionary Plastic-Coated and Double Capping Sealons!

and . . . beyond its responsibility for creating and manufacturing packaging for POSITIVE MILK PROTECTION, Sealright too has and continues to contribute merchandising and promotional programs on a nation-wide scale to help dairies and ice cream manufacturers sell more milk and milk products to America.

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term field trip on February 11. At Green Meadow Farms, club members saw a milking establishment for 480 cows. CHUCK GREEN, a former MSU dairy student, showed the group around the farm. In the afternoon, the club toured the MMPA plant at Ovid. This plant is used to handle the surplus milk produced in the Detroit market. Products manufactured at the Ovid plant include powdered milk, calf starter, and cottage cheese.

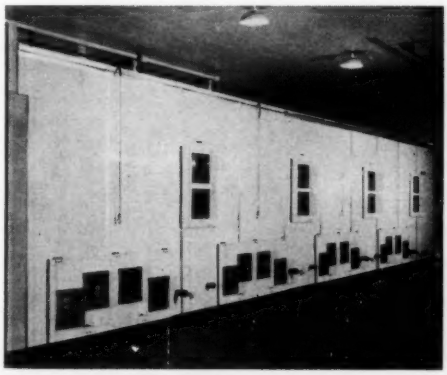
The MSU Dairy Club held its annual fitting and showing contest recently during Farmers' Week. Besides the fitting and showing contest, this year's program included a fitting demonstration together with a contest for FFA and 4-H members called What Do You Know About Dairy Cattle? FRANK GENDRON, 4-H dairy extension specialist, gave the fitting and showing demonstration. In the contest for FFA and 4-H members, each participant had to match six daughters with six dams, estimate the body weight of three cows, and estimate the milk produced in one day by three cows. Co-chairmen of this year's program were JOHN HUISJEN, senior from Fremont, Michigan, and DON USIAK, Shelby, Michigan, senior.

INDUSTRY NEWS

Dairyland Food Laboratories to Hold Open House at New Plant

An open house with guided tours through the new rennet manufacturing plant for visiting Dairy Science Association members and their families will be held June 7, 8, and 9, preceding the animal meeting, and June 15 and 16, following the meeting, by Dairyland Food Laboratories, Inc., Waukesha, Wisconsin.

This new plant, a modern and automated rennet manufacturing plant, is the result of 3 yr. of planning by PRESIDENT MERLE FARN-



Three sets of fiberglass tanks are used for extraction, finishing, and storing rennet.

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Eliminate lost batches, save time, speed production. Use *Numbered Blends* and the Dairy Laboratories system of rotation that supplies highest quality fresh culture *every week* to keep aroma, body, flavor and production uniform in all fermented milk products.

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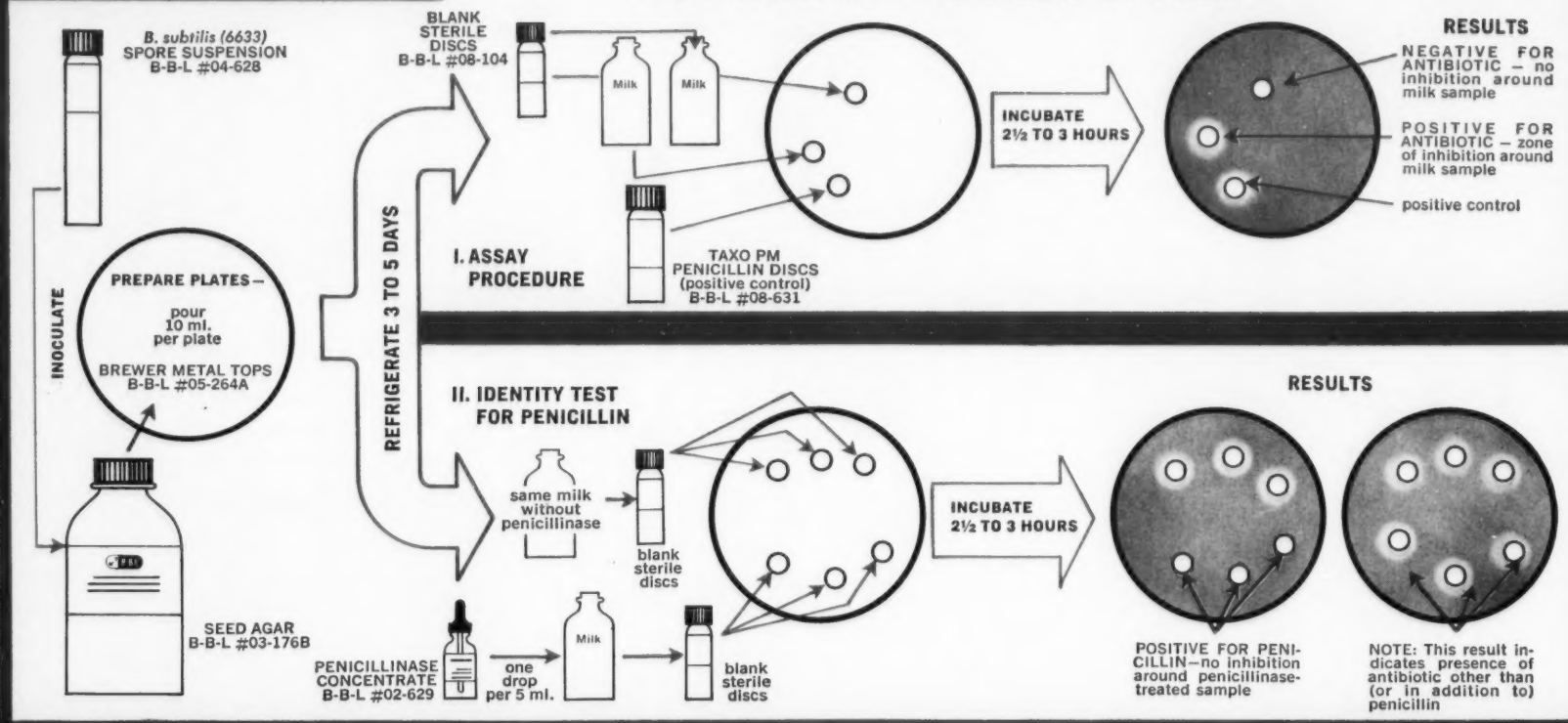
DETECTION OF PENICILLIN IN MILK



The presence of antibiotics in milk following mastitis therapy in cows has created serious public health problems and caused technical difficulties within the dairy industry. A rapid, practical laboratory procedure to assist regulatory agencies and the dairy industry in solving these problems was described by Arret and Kirshbaum.* This procedure employs rapid growth of a sensitive strain of *B. subtilis* for assaying the presence of antibiotics in milk and for determining its identity with penicillin. Inhibition of growth by the presence of as little as 0.05 unit of penicillin per ml. of milk sample is detectable within 2½ hours.

In answer to many requests for information about the availability of B-B-L products for this simplified procedure, the B-B-L Development Laboratory has prepared this TECHNICHART. It graphically illustrates the basic procedure, showing the materials necessary—**all of which are available from B-B-L.** A complete brochure with detailed technique and product listing is available upon request. No. 13

*Arret, B., and Kirshbaum, A.: J. Milk and Food Technol. **22**:329, 1959.

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B·B·L

HAM and RESEARCH DIRECTOR JOHN NELSON. The new plant, which began operations in January of this year, is the first installation of its kind to make use of fiberglass tanks and piping, fully automatic controls, and refrigerated tank jackets.

The new plant is equipped to process either flat-salted or dry-blown rennets. Three sets of large fiberglass tanks are used for extracting, finishing, and storing the rennet. Each set is fully enclosed within its own refrigerated jacket to maintain proper temperatures during processing. The refrigerated jackets completely eliminate the requirement for refrigerated manufacturing areas. Tank interiors are formed of molded white fiberglass and are completely seamless for ease of cleaning.

During the extraction cycle, preset controls are used to automatically operate a pump and valve system, which draws the salt brine on and off the rennets being processed. Transferring brine from one tank to another is accomplished by turning a switch at the large master control panel. All piping is constructed of noncorrosive fiberglass and is colored for identification. The sanitary system allows in-place cleaning of all piping. Taps on each tank permit sampling at each stage of processing.

The entire DFL plant will be open for guided tours from 7:30 A.M. to 5 P.M. during the period June 7 through 9 and June 15 and

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2. Arrange for his laboratory to perform special services that can only be handled by a lab staffed with chocolate technicians and equipped with the latest analytical and pilot plant equipment to produce special types of chocolate for ice cream.
3. Refer your problems to one of our consultants specializing in technical ice cream problems.
4. Conduct research on new ideas suggested by you.

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described by Nye and Knudtzon. This procedure employs rapid growth of a sensitive strain of *B. subtilis* for assaying the presence of antibiotics prepared this TECHNICHART. It graphically illustrates the basic procedure, showing the materials

16. No advance registration is required and both adults and children are welcome. The plant is located at 620 Progress Avenue, Waukesha, Wisconsin.

Beatrice Foods Promotes Salter

F. C. SALTER has been promoted to General Manager of Cream Procurement and Butter Manufacturing for Beatrice Foods Co.

F. C. Salter

He has been assistant general cream procurement manager since 1955, has 35 yr. of service with the company, and is experienced in every phase of creamery operation. He joined the company in 1925 as a general employee in a butter manufacturing plant in Centerville, Iowa.

In 1935, he was advanced to the position of manager of the procurement department at Des Moines, Iowa, and in 1947, he was named manager of the Fort Madison, Iowa, Plant. From 1950 until 1955, he was manager of the company's creamery plant at Galesburg, Ill.

Foremost Dairies Elects Rankin

J. L. RANKIN, executive vice-president and director, was elected president and chief executive officer of Foremost Dairies, Inc., at a meeting of the Board of Directors held in New York following the annual meeting of stockholders. P. E. REINHOLD continues as chairman of the board and G. D. TURNBOW, president of Foremost since 1953, becomes chairman of the executive committee. Both will remain active in the company.

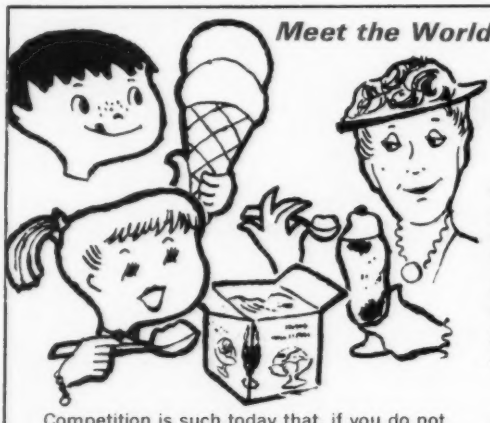
J. L. Rankin

Foremost is one of the major dairy products companies in the world, serving more than 2,300 communities in the United States and distributing its products in 40 countries overseas.

Dairy Technology Societies

Central Illinois—March 8th meeting was held at the Blue Mill Restaurant, Decatur. The program was a panel presentation: Facts

Meet the World's Best Judges of Ice Cream . .



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Only your customers will determine whether your special ice cream flavor is a good selection and seller. Will they come back for more? Will they tell others how good it is? They alone, your customers, will judge the goodness and quality. Look for your answers in your sales analysis report.

*with due respect to your flavor panel

Competition is such today that, if you do not offer your customers what they want and like, they will buy elsewhere. You cannot afford to lose that business. To avoid disappointment and to insure a successful and profitable program, let us help you with the many selections we have to offer.

In the interest of the ice cream manufacturer we have developed the unique RAMSEY PLAN FOR MERCHANDISING ICE CREAM which offers you a guide and a wide selection for a successful and profitable program. Our contribution includes the presentation of ice cream samples for your ready-to-taste appraisal.

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BACK NUMBERS OF JOURNAL NEEDED

Certain numbers of the Journal of Dairy Science are needed to maintain an inventory and to take care of orders. If any of the numbers listed are available and of no further use to the owners, please send them to the Garrard Press, 510 North Hickory Street, Champaign, Illinois. Fifty cents plus postage will be paid for each number.

1919—March, May, July, September, and November
1920—March, July
1921—July, September
1923—January, March, May, July
1925—September
1926—January
1932—September
1943—April
1959—January, April
1960—October

There are less than five issues of the following in inventory:

1920—January, May, September, November
1921—January, May, November
1922—January
1923—September, November
1924—January, May, July
1925—January, July
1926—March, May, November
1928—January
1931—May
1932—July, November
1938—December
1946—January, March, April
1947—March
1955—March

About Plastic Containers for Bulk Dispensing of Milk. On the panel: H. F. Cox, Jr., Manager, Polygal Company, Indianapolis, Ind.; G. F. Kahle, vice-president Sales, Scholle Container Corporation, North Lake, Illinois; G. F. Dammann, Production Manager, Sealtest Foods Company, Peoria; and Vernon Jones, Manager, Beatrice Foods Company, Mattoon.

April 12th meeting was in Peoria, with Joe Petersen, Director Quality Control, The Borden Company, Central Division, speaking on Cottage Cheese Manufacture and Problems Associated with Manufacturing. May 10th meeting will be at the Champaign Country Club.

Central Michigan—The March meeting was held at the Meadow Gardens. Speaker for the evening was Dr. G. Malcolm Trout, on Problems in Quality Control.

Detroit—Regular monthly meeting was March 13 at Cregar's Pickwick House on Grand River. James Warner, State Representative, spoke on important legislative matters of general and specific interest to the dairy industry.

Kansas—Two closely allied subjects were discussed at the March 13 meeting—The Circulating Parts Washer Comes of Age and Designing and Building Milk Processing Equipment to Be Cleaned-in-Place. The speaker was Arthur Whitehouse, Girton Manufacturing Company, Millville, Pennsylvania.

Kansas City—Problems with Bulk Tank Extraneous Matter was the subject discussed by Harmon Johnson, Western Division Manager, Filter Products Department, Johnson & Johnson Company, Salt Lake City, Utah, at the group's March 23rd meeting at the Black Angus Restaurant.

Metropolitan—Dr. James A. White, Professor of Dairy Industry, Cornell University, Ithaca, New York, spoke at the March 13th meeting, discussing Milk, A Materials Handling Problem.

Tri-State—Two speakers, using film to illustrate their subject matter, featured the March 22nd meeting. The general subject was The Pure-Pak Plastic Milk Container, Henry Breejen, Sales Engineer, Ex-Cell-O Corporation speaking on Operation of Plastic Machines, and Bob Anniek, Production Coordinator for Plastic Cartons, International Paper Company, on How Plastic Paperboard Is Prepared.

Western Michigan—Finger's Cafe, Grand Rapids, was the locale of the March 20th meeting. Guest speaker was Frank Koval, Secretary of the Michigan Dairy Products Association, on the topic Current Dairy Legislation.

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RESEARCH PAPERS

STARTER METABOLISM. V. THE MECHANISM OF ACETOIN FORMATION AS DETERMINED WITH C^{14}-LABELED SUBSTRATES [1, 2]

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SUMMARY

C^{14}-labeled substrates, including uniformly labeled glucose and citric acid, 1-5-labeled citric acid, and pyruvates labeled in three different positions were used to determine the source of carbon and mechanism of formation of acetoin by a mixed starter culture consisting predominantly of *Streptococcus cremoris* and *Leuconostoc* species.

The starter culture was found to transform the C^{14} from Carbon Atoms 2, 3, 4, or 6 of citric acid into the acetoin molecule; whereas, Carbon Atoms 1 and 5 were not.

The addition of uniformly labeled glucose to a dialyzed milk medium containing either unlabeled citrate or pyruvate resulted in labeled acetoin, as did the addition of uniformly labeled citric acid to the dialyzed milk medium containing unlabeled glucose.

The methyl and alpha-keto carbons, but not the carboxyl carbon, of pyruvic acid were transformed into acetoin. Carboxyl carbon appeared in the CO_2.

A possible pathway for the formation of acetoin is proposed.

According to many investigators, acetoin is formed from citric acid in mixed strain starter cultures. However, Virtanen (19) and Coppens (3) believed that acetoin was formed from lactose and that citric acid was needed as a hydrogen acceptor. Until recently, positive indication of the origin of the carbon skeleton for the acetoin molecule was lacking.

The work by DeMan (4), using buffered cell suspensions of *Betacoccus cremoris*, and by Mizuno and Jezeski (11), using a mixed strain starter culture in a dialyzed milk medium, indicated that acetoin could be formed from citric acid in the absence of fermentable sugars; also, alpha-acetolactic acid decarboxylase activity was demonstrated in the organisms studied. Other work (7, 11) has indicated that acetoin may be formed from citric, oxalacetic, and pyruvic acids. However, it was found that a number of the tricarboxylic acid cycle intermediates such as cis-aconitic, alpha-keto glutaric, succinic, and malic acids did not stimulate acetoin formation (11).

There is some question as to the intermediate carbon transformations leading to the formation of acetoin; and the objective of this study was to study further the mechanism of the formation of acetoin and the carbon source(s) from which it is formed.

Received for publication November 18, 1960.

[1] Paper No. 4515, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Taken from data submitted to the graduate faculty of the University of Minnesota by the senior author in partial fulfillment of the requirements for the Ph.D. degree.

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MATERIAL AND METHODS

The chemical procedures, for the most part, were previously described, as were the bacteriological procedures and the methods for the preparation of the reconstituted nonfat dry milk solids medium (NDMS) and the dialyzed milk medium (11).

Separation of fermentation products. Ether extraction was employed for the separation of neutral compounds and acids in the fermentation mixture. Since the fermentation mixture (dialyzed milk medium) usually had a pH of 4.6 or lower, the precipitated milk proteins were removed by a preliminary centrifugation. The mixture was then clarified with zinc sulfate by the method of Somogyi (16). A continuous ether extractor similar to that described by Neish (13) was used. The clarified fermentation solution was extracted for 48 hr. to remove the neutral compounds, then acidified with hydrochloric acid and re-extracted for 30 hr. with fresh ether to obtain the organic acids. The neutral fraction was assayed for acetoin-diacetyl by the method of Van Niel (18) and the acid fraction was titrated with standardized aqueous sodium hydroxide to the phenolphthalein end point. The sample was then evaporated to dryness; the residue was redissolved and an aliquot was acidified and then steam-distilled to separate the volatile from the nonvolatile acid(s), according to the method of Neish (13). The distillate was titrated with sodium hydroxide, evaporated to dryness, and saved for the radioactivity assay.

Isotopic analysis. In most cases, the radioactivity assays were made by converting the sample to barium carbonate by a modification of the wet combustion procedure described by Calvin *et al.* (2), in which the Van Slyke-Folsh combustion solution consisting of chromic trioxide, phosphoric acid, and fuming sulfuric acid (the potassium iodate omitted) was used.

A Nuclear Instrument Company scaler, Model 165, equipped with a Geiger-Mueller tube having a thin mica end-window (1.4 mg/cm^2) was used for all C^{14} assays. The sample was prepared for counting by spreading a 5-mg. sample evenly over an area of 3.14 cm.^{2} on a previously dried aluminum planchet.

Corrections were not made for back-scattering or self-absorption because, in nearly all experiments, counts were made on a barium carbonate film of relatively uniform thickness and under fixed geometry.

Isotopically labeled compounds. Uniformly labeled glucose-C^{14},[4] citric acid 1, 5-C^{14},[5] and sodium pyruvates[6] 1-C^{14}, 2-C^{14}, and 3-C^{14} were obtained from commercial sources.

The uniformly labeled citric acid was not commercially available and was prepared by a mold fermentation method, using uniformly labeled sucrose-C^{14}[7] as the sole source of carbon. The strain of *Aspergillus fonsecaeus* used for this fermentation was kindly furnished by Dr. S. G. Knight of the University of Wisconsin. The general procedure used for this fermentation was one described

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[5] Tracerlabs, Inc., Boston 10, Massachusetts.
[6, 7] Nuclear Instrument and Chemical Corporation, Chicago 10, Illinois.

by Doelger and Prescott (5). A pH of 2.4 was optimal for this strain of mold under the conditions of the fermentation. The solubility of atmospheric CO_2 at this pH is very low and, consequently, the fixation of atmospheric CO_2 was not expected to occur.

Although the citric acid produced by this procedure would be expected to be uniformly labeled, it was subjected to a three-step degradation by the method of Weinhouse *et al.* (21). While not a complete degradation, the results of this work, summarized in Table 1, indicate a uniformity of labeling so far as the

TABLE 1

Summary of the results on degradation of citric acid–C^{14}

Position of C-atom in citric acid molecule	m*M* CO_2 recovered as $BaCO_3$	Total ct/min/ total sample	Spec. act. ct/min/m*M* $BaCO_3$
6	0.47	4,640	9,900
1, 5	1.03	10,900	10,600
2, 3, 4	1.46	14,800	10,100
Total	2.96	30,340	
Expected	3.00	29,000	
Per cent recovered	97.5	104	

degradation was carried out. In this procedure, the Carbon Atom 6 of the citric acid molecule was split off with sulfuric acid at 25° C., and the CO thus formed was converted to CO_2 by passage over heated iodine pentoxide. Next, Carbon Atoms 1 and 5 of the acetone dicarboxylic acid residue were split off with sulfuric acid at 100° C. Finally, the acetone residue corresponding to the Carbon Atoms 2, 3, and 4 of the citric acid molecule was completely degraded by the wet combustion method. All of the radioactivity counts were made from barium carbonate.

EXPERIMENTAL RESULTS AND DISCUSSION

Uniformly labeled glucose-C^{14}. This compound was appropriately diluted with untagged glucose and added to the dialyzed milk medium (13.9 m*M* glucose-C^{14}, specific activity of 15,600 ct/min/m*M*, to 250 ml. of medium), which already contained unlabeled sodium pyruvate or citric acid; the medium was adjusted to pH 5.0 with 2.0 *M* phosphoric acid. Preliminary experiments indicated that glucose alone did not yield acetoin, so that either citric or pyruvic acid had to be added.

The results of this experiment are summarized in Table 2. These data indicate that when glucose-C^{14} and unlabeled pyruvate were added to the medium, the specific activity of the resulting acetoin was 2,850 ct/min/m*M*; the specific activity of the CO_2 was 775 ct/min/m*M*, whereas that of the added glucose was 15,600 ct/min/m*M*. On the basis of ct/min/m*M* of carbon, it may be observed that the specific activity of the CO_2 is of the same order as that calculated for acetoin. While the specific activities of the acetoin and CO_2 were considerably lower than that of the added glucose, the data definitely indicate that a part of

the carbon skeleton for the acetoin was derived from glucose. Similar results were obtained when unlabeled citric acid was added to the medium containing uniformly labeled glucose-C^{14}. Previous studies (11) indicated that acetoin was not formed to any appreciable extent in the presence of glucose alone. Hence, a likely explanation for the observations made using uniformly tagged glucose is that both the glucose and the citric acid form a common intermediate, namely pyruvate, and that there is a mixing of the isotopically tagged pyruvate and untagged pyruvate which is then transformed to acetoin.

TABLE 2

Tracing the source of carbon for acetoin with uniformly labeled glucose-C^{14}

Compound	m*M* compound	Total ct/min	Spec. act. ct/min/m*M*
Glucose-C^{14} added	13.9	218,000	15,600
Sodium pyruvate added	1.75	....	....
Acetoin recovered	0.18	520	2,850
CO_2 recovered	0.93	720	775
Glucose-C^{14} added	13.9	218,000	15,600
Citric acid added	1.31	....	....
Acetoin recovered	0.26	560	2,150

Fermentation conditions. Medium: Dialyzed milk with sodium pyruvate, or citric acid added to force the production of acetoin. Incubation temperature and time: 21-22° C. for 20-24 hr. Medium adjusted to pH 5.0 with 2.0 *M* H_3PO_4.

Citric Acid-1, 5-C^{14}. The dissimilation of citric acid-1, 5-C^{14} with respect to acetoin formation was studied in a manner similar to the previous experiment, except that unlabeled glucose and the labeled citric acid were added to the medium. From the data summarized in Table 3 it will be observed that the

TABLE 3

Tracing the source of carbon for acetoin with citric acid–1, 5-C^{14}

Compound	Total ct/min	m*M* compound	Spec. act. ct/min/m*M*
Citric acid–1, 5-C^{14} added	202,000	1.95	103,000
Glucose added	....	13.9	....
Acetoin recovered	320	0.56	570
CO_2 recovered	42,500	3.2	13,300

Fermentation conditions. Medium: Dialyzed milk medium. Incubation temperature and time: 21-22° C. for 20-24 hr. Medium adjusted to pH 5.0 with 2.0 *M* H_3PO_4.

specific activity of the added citric acid was 103,000 ct/min/m*M*, whereas that of the acetoin recovered was 570 ct/min/m*M*, and that for the CO_2 was 13,300 ct/min/m*M*.

These results indicate that not more than traces of the two terminal carbon atoms of the citric acid molecule were transformed into the acetoin. Only about 0.15% of the C^{14} of the citric acid 1, 5-C^{14} was found in the acetoin, and the specific activity of the acetoin was roughly 0.005 of that of the citric acid, whereas approximately one-fifth of the C^{14} added appeared in the CO_2.

These results would suggest that either the carbon atoms for the formation of acetoin are not derived from the citric acid or only from the carbon atoms numbers 2, 3, 4, and/or 6 of the citric acid molecule. Any scheme for the transformation of citric acid into acetoin must take this fact into consideration.

Uniformly labeled citric acid-C^{14}. In an experiment similar to the previous one, uniformly labeled citric acid was used instead of the citric acid-1, 5-C^{14}, in order to further study the transformation of citric acid to acetoin. These data summarized in Table 4 show that C^{14} from the uniformly labeled citric acid appeared in acetoin, volatile acid(s), nonvolatile acid(s), and CO_2.

TABLE 4

A study of the dissimilation of uniformly labeled citric acid

Fraction	Total ct/min	m*M* compound	Spec. act. ct/min/m*M*	Spec. act. ct/min/m*M* of carbon
Citric Acid-C^{14}	213,000	3.91	54,600	9,100
CO_2 recovered	23,900	3.92	6,100	6,100
Acetoin recovered	24,600	1.12	21,800	5,500
Total acid recovered	148,000	25.6	5,800	....
Volatile acid recovered	76,900	5.76	13,300	....
Lactic Acid (Calculated = total acid − volatile acid)	71,100	19.8	3,580	1,200

Fermentation conditions. Medium: Dialyzed milk containing 1.0% glucose. Incubation time and temperature: 21-22° C. for 20-24 hr. Medium adjusted to pH 5.0 with 2.0 *M* H_3PO_4.

$$\text{Spec. act., ct/min/m}M\text{ carbon} = \frac{\text{spec. act., ct/min/m}M\text{ of compound}}{\text{No. of carbon atoms in molecule}}.$$

On the basis of total counts per minute, the total acids recovered had the highest count of 148,000 ct/min; this figure indicates that about 70% of the citric acid–carbon was converted to the acids. Of this, the volatile acid fraction had a total count of 76,900 ct/min, indicating that about 36% of the citric acid was converted to volatile acid. Acetoin had a total count of 24,600 ct/min, indicating that about 11.5% of the citric acid was converted to acetoin; and CO_2 had a total count of 23,900 ct/min, indicating that about 11% of the citric acid was converted to CO_2. The total recovery of C^{14} was 92.5%. The remaining 7.5% unaccounted for probably was to be found in the ethanol (not assayed) or was incorporated in the cell substances.

On the basis of specific activities per compound, acetoin had the highest value of 21,800; the volatile acids had the second highest value of 13,300; CO_2 had the third highest value of 6,100, and nonvolatile acid (predominantly lactic) had the lowest value of 3,580.

The last column of Table 4 is a calculated value obtained by dividing the specific activity per compound by the number of carbon atoms in the molecule. These figures give a relative concentration of C^{14} in the various compounds. For example, an inspection of the data indicates that the specific activity per carbon atom for acetoin is about two-thirds of the value for citric acid-C^{14}. This would indicate that about two-thirds of the carbon for the formation of acetoin

was derived from the citric acid. The remaining one-third presumably came from the glucose by way of pyruvic acid. A similar interpretation could be made for the other end products. Theoretically, if citric acid were the sole source of carbon atoms for a particular compound, the specific activity on a carbon basis should be identical with that of the added citric acid.

Sodium pyruvate-C^{14}. The formation of acetoin from pyruvate was studied by adding, in separate experiments, sodium pyruvate-C^{14} labeled in the carboxyl, alpha-keto, and methyl carbon atoms, respectively, to the dialyzed milk medium containing 1.0% glucose and adjusted to pH 5.0 with phosphoric acid. The data summarized in Table 5 show that when sodium pyruvate-1-C^{14} was

TABLE 5

A study of the formation of acetoin using pyruvic acid-C^{14}

	Total ct/min	m*M* compound	Spec. act. ct/min/m*M*
Dissimilation of sodium pyruvate-1-C^{14}			
Na pyruvate-1-C^{14} added	112,800	2.27	49,700
Acetoin recovered	13	0.39	33
CO_2 recovered	4,600	0.75	6,130
Dissimilation of sodium pyruvate-2-C^{14}			
Na pyruvate-2-C^{14} added	47,500	2.25	20,900
Acetoin recovered	9,900	0.49	20,100
CO_2 recovered	12	0.69	18
Dissimilation of sodium pyruvate-3-C^{14}			
Na pyruvate-3-C^{14} added	86,500	2.27	38,000
Acetoin recovered	9,000	0.36	25,000
CO_2 recovered	3	0.71	4

Medium: Dialyzed milk containing 1.0% glucose. Medium adjusted to pH 5.0 with 2.0 *M* H_3PO_4. Incubation temperature and time: 21-22° C. for 20-24 hr.

added to the medium, the resulting acetoin was essentially untagged; but the CO_2 was relatively heavily tagged, thus indicating that the carboxyl carbon of the pyruvate showed up in the CO_2 but not in the acetoin. When the sodium pyruvate-2-C^{14} was added, the C^{14} appeared in the acetoin but not in the CO_2, indicating that the alpha-keto carbon of the pyruvate was transformed into acetoin but not to CO_2. When sodium pyruvate-3-C^{14} was added, the results were similar to those obtained with sodium pyruvate-2-C^{14}, indicating that the methyl carbon of the pyruvate was transformed into acetoin but not into CO_2. Collectively, these studies show that the alpha-keto and methyl carbons of the pyruvate are transformed into acetoin, whereas the carboxyl carbon is not transformed into acetoin but is converted to CO_2. Any mechanism for the transformation of pyruvate into acetoin must take these facts into consideration.

DISCUSSION

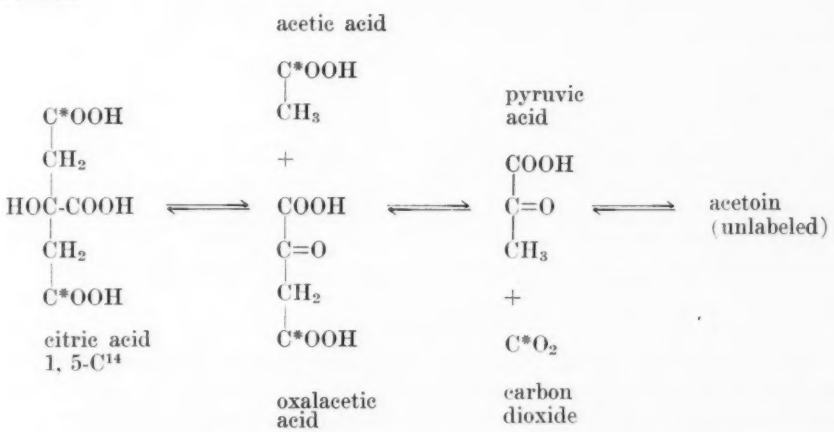
Previous work (4, 7, 11) has shown that citric, oxalacetic, and pyruvic, as well as alpha-acetolactic acids, may promote the formation of acetoin. Intermediates of the TCA cycle such as cis-aconitic, alpha-keto glutaric, succinic, and malic acids do not stimulate the formation of acetoin (11) and, thus, the

possibility that a reversal of the TCA cycle would lead to the formation of acetoin seems improbable.

To reconcile the above observations, it must be assumed that the initial cleavage of the citrate molecule is a C_2-C_4 split by way of either a reversal of the condensing enzyme of Stern and Ochoa (17) or the citrate-splitting enzyme of Wheat and Ajl (22, 23). This is supported by the observation that oxalacetate gives rise to acetoin and, further, that some of the pyrolytic products of citric acid (1, 15) do not.

The oxalacetic acid formed from citric acid is then probably decarboxylated to form pyruvic acid. In turn, the pyruvic acid is decarboxylated to form a hypothetical active acetaldehyde, which then condenses with a molecule of pyruvic acid to form alpha-acetolactic acid. Finally, the alpha-acetolactic acid is decarboxylated to form acetoin.

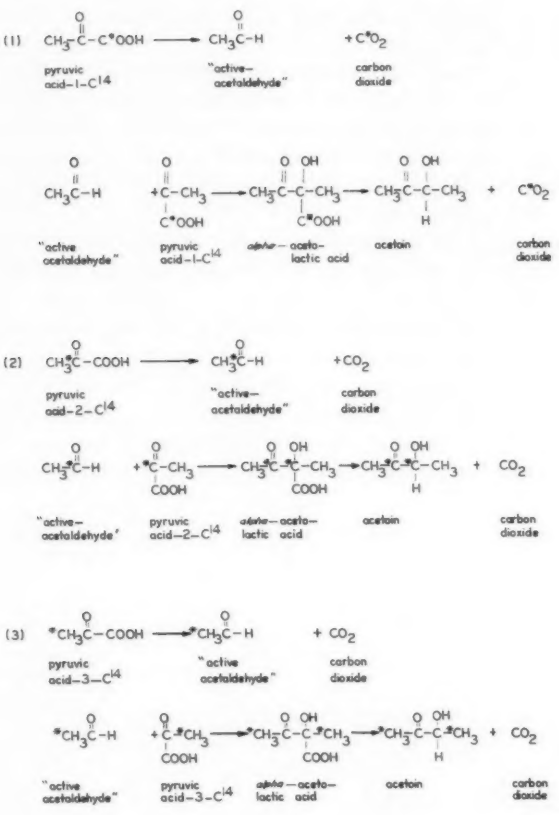
Evidence for such a pathway is to be found in the mode of breakdown of the labeled citric and pyruvic acids. The C^{14} from the bio-degradation of citric acid–1, 5-C^{14} was found in the CO_2 but not in the acetoin. The following scheme of conversion of citric acid to acetoin would support these experimental results.



Such a scheme would also account for the uniformly labeled citric acid–C^{14} giving rise to the acetoin-C^{14}.

The pyruvic acid-C^{14} studies indicated that the methyl and alpha-keto, but not the carboxyl, carbon atoms were transformed into the acetoin molecule; the latter appeared in the CO_2. This experimental evidence, together with the fact that alpha-acetolactic acid decarboxylase activity was demonstrated in the *Leuconostoc* species (4, 11), supports the theory that pyruvic acid is converted to the acetoin by way of alpha-acetolactic acid. Juni (9), Moat and Lichstein (12), Watt and Krampitz (20), and others have presented evidence for the existence of alpha-acetolactic acid in various species of bacteria.

The mechanism for this reaction and an accounting of the C^{14} in the variously labeled pyruvic acids are given by the following equations:



These equations can be further verified by degrading the acetoin by the methods described by several authors (2, 8, 10), to determine the position of the labeling. The preliminary results of Sandine *et al.* (14), which indicated that *S. diacetilactis* could produce CO_2 from one or more carboxyl carbons of citric acid, are compatible with the data and discussion presented in this paper.

Although the existence of an active acetaldehyde has not yet been demonstrated, there are several evidences which support its existence. Free acetaldehyde failed to stimulate the formation of acetoin, according to a previous study (11). Juni (9), likewise, has found that acetoin was not formed from free acetaldehyde by other bacterial enzymes. This is interpreted to mean that in the formation of acetoin from pyruvic acid via alpha-acetolactic acid, the two-carbon moiety which condenses with pyruvic acid is not free acetaldehyde but must be in nascent or active form. Diphosphothiamine dependency was interpreted to mean that a possible diphosphothiamine-active aldehyde complex exists, according to Dolin (6).

Finally, the fact that uniformly labeled glucose-C^{14} yielded labeled acetoin in the presence of unlabeled pyruvic or citric acid, whereas glucose alone yielded little or no acetoin, would indicate that there is a mixing of a common precursor of acetoin. This common precursor is probably pyruvate, which can be derived from glucose by way of the Meyerhof-Embden scheme of glycolysis or from citric acid by the C_2-C_4 split.

The fact that oxalacetate gives rise to acetoin may be taken as evidence that a C_2-C_4 split does occur. As to the mechanism of this split and/or the enzyme involved, there is little basis for conclusion from these experiments. There is the possibility that the citrate-splitting enzyme (22, 23) is active, based on the inhibitor studies previously reported (11), where acetoin production was not materially affected by a sulfhydryl inhibitor. However, the pH optimum for the citrate-splitting enzyme appears to be in the vicinity of pH 7.0, and previous work (11) showed active acetoin production in the vicinity of pH 5.0 and scarcely none at pH 7.0. Furthermore, in cell-free systems, this enzyme appears to be inhibited by oxalacetate and acetate.

It should be recognized that pH effects on individual metabolic reactions are best demonstrated in isolated systems, and that it may not be valid to extrapolate such findings to systems containing intact cells in complex environments. Therefore, in the absence of data from more critically designed experiments, the significance of the effect of pH on acetoin production may be the result of the interaction of several factors which have not been adequately evaluated or controlled.

Finally, where there are natural associations of organisms, such as in starter cultures, results of studies on isolated enzyme systems may not be applicable because of the interactions between species in their metabolic activity. What on the surface may seem to be only fortuitous association may, in reality, be true interdependence with respect to certain metabolic requirements and functions.

ACKNOWLEDGMENT

The substantial contribution of Economics Laboratory, St. Paul, Minnesota, toward the completion of this research is gratefully acknowledged. Laboratory facilities and equipment were furnished to the senior author to make possible the work reported in this paper and in the previous publication (11).

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CHROMATOGRAPHY OF MILK PROTEINS ON ANION-EXCHANGE CELLULOSE [1]

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SUMMARY

A rapid and highly reproducible method of fractionating milk proteins on anion exchange cellulose (DEAE) is described. Gradients of NaCl concentration were employed for elution in phosphate buffer between pH 7.0 and pH 6.6. Separation of the whey protein fraction from the casein fraction by DEAE cellulose chromatography was complete and clear-cut, without overlapping peaks. Whey protein components were eluted in the range of 0 to 0.2 *M* NaCl. Casein components began to emerge from the column when the concentration of NaCl in the eluting buffer exceeded 0.2 *M*. Ten, and possibly 11, chromatographic components were obtained from the casein fraction, and at least eight from the whey fraction.

The method can be used for identifying components in a mixture of proteins, as was illustrated by chromatographic identification of β-lactoglobulins. By chromatographing purified preparations of α-lactalbumin, α-casein, β-casein, κ-casein, and lactoglobulin their positions on the chromatogram of dialyzed skimmilk were identified. The resolving power obtained was higher than with conventional electrophoresis and, furthermore, it appears to be superior to other methods in establishing the kind and extent of contamination of a given preparation with other protein components. The method allows isolation of protein components of milk under very mild conditions, and should be especially useful for isolating and purifying milk protein components when enzymatic and other biological activities are to be preserved.

Cellulose ion exchangers for the fractionation and purification of proteins were introduced by Sober and co-workers (14, 19, 22), and their use was reviewed by Sober and Peterson (20, 21).

Ion exchange cellulose has proved particularly useful for fractionation of the mixture of closely associated proteins such as blood serum proteins (22) and egg white proteins (15). Its resolving power is greater than that of conventional electrophoresis. Adsorbed protein entities can be eluted under very mild conditions. Enzymes of pancreatic juice have been fractionated and isolated with preservation of their enzymatic activities (10). Relatively large amounts of proteins can be fractionated with nitrogen recovery that is nearly quantitative (22).

Preliminary work (28) on fractionation of milk proteins by anion exchange cellulose DEAE-SF (Diethylaminoethanol on Solka-Floc cellulose lattice) indicated that a good resolution can be obtained, provided that the conditions with respect to the pH, nature, and concentration of the buffer and eluant are satisfied. Schober *et al.* (17) reported fractionation of whey proteins into eight distinct fractions, with DEAE cellulose by elution with ammonium acetate buffers.

Received for publication June 11, 1960.

[1] This study was supported in part by funds from the California Dairy Industry Advisory Board.

The present paper describes the methods of fractionating milk proteins on DEAE cellulose by both stepwise and gradient elutions, obtaining fractions suitable for analytical and preparatory purposes, and identifying the major components of casein and whey proteins on the elution diagrams.

EXPERIMENTAL PROCEDURE

Preparation of the sample. Fractionation of milk proteins on DEAE cellulose was studied on the following samples: skimmilk, purified isoelectric casein, acid whey, commercial β-lactoglobulin, and prepared samples of α-lactalbumin, α-casein, β-casein, κ-casein, and lactoglobulin fraction.

The milk was from Holstein cows and separated immediately after milking. The separated skimmilk, cooled to 2-5° C., was dialyzed in cold for two days against large amounts of starting buffer, 0.02 *M* sodium phosphate at pH 7.0. The buffer was agitated with a magnetic stirrer and changed every 12 hr.

The casein and whey protein fractions were prepared from another portion of the original skimmilk. The casein was washed four times with distilled water and twice redissolved and reprecipitated with acid (6). The final preparation was redissolved with dilute NaOH, raising the pH gradually, with constant agitation, up to pH 8.5. The casein preparation was then dialyzed by a procedure similar to that used in the dialysis of skimmilk.

The acid whey from casein precipitation was kept overnight in cold at pH 4.6, and then filtered. Filtered whey was adjusted to pH 6.8 with dilute NaOH, and the traces of casein were reprecipitated by acidification to pH 4.6. The whey was filtered, adjusted to pH 7.0, and dialyzed as described above.

The other samples were prepared as follows: β-lactoglobulin [2] was a commercial sample. Alpha-lactalbumin, α-casein, and β-casein were supplied by Dr. R. Jenness.[3] Alpha-lactalbumin was prepared by the method of Gordon and Ziegler (5) and recrystallized four times. The casein fractions were prepared from a single lot of casein by the urea method of Hipp *et al.* (7). The α-casein was precipitated five successive times from 4.63 *M* urea and then dissolved in water, reprecipitated at the isoelectric point, washed, and freeze-dried. Electrophoresis in veronal buffer, at pH 7.8, $\mu = 0.1$, 4° C., 7,200 sec., showed a single peak. The β-casein was precipitated three successive times between 3.3 *M* and 1.7 *M* urea at pH 4.7, washed twice with 1.7 *M* urea and five times with water, and freeze-dried. Electrophoresis in veronal buffer at pH 8.6, $\mu = 0.1$, 4° C., 7,200 sec., showed a small amount (3-5%) of material moving at the mobility of α-casein.

About 70% pure kappa-casein fraction was supplied by Dr. C. A. Zittle.[4] It was prepared by treating whole casein with 0.5 *M* $CaCl_2$ and precipitating the calcium-sensitive casein. The crude kappa-casein remaining in solution was precipitated with acid and dried.

For application on DEAE column β-lactoglobulin and α-lactalbumin were

[2] Obtained from Nutritional Biochemical Co., Cleveland, Ohio.

[3] Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota.

[4] Eastern Utilization Research and Development Div., USDA, Philadelphia, Pa.

dissolved separately in starting buffer and dialyzed. Casein component preparations were treated in a similar manner, except that they were dissolved in starting buffer adjusted with NaOH to pH 8.5-8.6.

The dialyzed preparations were then ready for chromatographic analysis.

Preparation of the adsorbent column. Anion exchange cellulose adsorbent DEAE-SF [5] was used. The cellulose was first soaked in 1 *N* NaOH to ensure complete regeneration. The adsorbent was washed with large volumes of distilled water to remove the NaOH, and then with several volumes of 0.02 *M* Na-phosphate buffer to equilibrate to pH 7.0. The resulting suspension was allowed to stand for 30 min. to let the larger cellulose particles settle. The adsorbent remaining in suspension was discarded. The suspension of sedimented particles (dry weight 17 to 22 g.) was then poured as a slurry into a glass chromatographic tube (30 by 5.5 cm.) fitted at the bottom with a coarse fritted disc covered with a filter paper–glass wool–filter paper layer. The adsorbent was allowed to settle under flow conditions induced by gravity, and a filter paper–glass wool–filter paper layer was placed on the top of the column to prevent erosion by the incoming buffer solution. A device described by Jennings (9) to control channeling, voids, and disruption of pressure-developed chromatographic columns was then applied, and the adsorbent was further compacted

[5] Obtained from Bio-Rad Laboratories, Richmond, California.

TABLE 1
Schedule of eluting solutions

Buffer No.	Phosphate	NaCl	NaOH	pH [d]	Volume		
						Gradient	
					Stepwise [c]	Whey [a] proteins	Casein [b]
	(M)	*(M)*	*(N)*		*(ml.)*	*(ml.)*	*(ml.)*
1	0.02	0	0	7.00	200	340	500
2	0.02	0.050	0	6.97	200	240	0
3	0.02	0.075	0	6.95	200	240	0
4	0.02	0.100	0	6.93	200	240	0
5	0.02	0.125	0	6.90	200	240	0
6	0.02	0.150	0	6.88	200	240	0
7	0.02	0.175	0	6.86	200	240	0
8	0.02	0.200	0	6.84	200	240	0
9	0.02	0.225	0	6.82	200	240	400
10	0.02	0.250	0	6.80	200	240	400
11	0.02	0.275	0	6.79	200	240	400
12	0.02	0.300	0	6.78	200	240	400
13	0.02	0.325	0	6.77	200	0	400
14	0.02	0.350	0	6.75	200	0	400
15	0.02	0.500	0	6.68	200	300 [c]	400
16	0.02	1.000	0	6.52	200	0	400
17	0	0	0.1	.....	200	.....	.....
18	0	0	0.2	.....	200	.....	.....
19	0	0	0.7	.....	200	.....	.....
20	0	0	1.0	.....	200	.....	.....
Total					4,000	3,280	3,700

[a] With two mixing chambers.
[b] With one mixing chamber.
[c] Applied directly on the column.
[d] Apparent pH measured by Beckman Model G pH meter.

by nitrogen pressure at 5-6 p.s.i. until column height was constant. The column was mounted above a fraction collector and washed with 0.02 *M* Na-phosphate buffer at pH 7.0 to attain pH equilibrium.

Preparation of buffer. Phosphate buffer of pH 7.0 was prepared by mixing 5.75 liters of 0.02 *M* Na_2HPO_4 and 4.25 liters of 0.02 *M* of Na $H_2PO_4H_2O$. Final pH was adjusted to pH 7.0 ± 0.05. The NaCl elution buffers were prepared by dissolving the corresponding amounts of NaCl (Baker analyzed Reagent) in the phosphate buffer to give a range of NaCl concentration from zero to 1.00 *M* (See Table 1).

Chromatography. Two methods of elution were used: stepwise changes of the buffer, and gradient elution. These methods have been compared (15, 20, 21).

In stepwise elution, the dialyzed preparations were applied on the DEAE column (6-7 by 5.5 cm.) and washed into the adsorbent with several portions of the starting buffer. Stepwise change in NaCl concentration with corresponding change in pH was used to elute the proteins. Flow rates in the order of 1,000 ml/hour were obtained with a nitrogen pressure of about 5 p.s.i. The effluent was collected by fraction collector with a volumetric siphon (capacity 20 ml.) and examined in a Beckman DU spectrophotometer at 280 mμ.

In the gradient elution, the dialyzed protein preparations were applied on the column (6-7 by 5.5 cm.) and washed with 100 ml. of starting buffer, in 20-ml. portions. Figure 1 shows the scheme of the gradient elution used. In this work, one or two mixing chamber systems were employed. The advantages of using two or more mixing chambers have been discussed by Bendich *et al.* (3). The adsorption column is connected with tygon tubing to Chamber 1, containing starting buffer (0.02 *M* Na phosphate of pH 7.0). Chamber 1, in turn, is connected with one or more chambers in series, containing the same buffer and NaCl in increasing concentrations. As the buffer in mixing Chamber 1 flows into the column, the next buffer automatically flows into the chamber because of the slight vacuum created by the reduction in volume of the starting buffer. Thus, the amount of buffer in the mixing chambers is always constant. When the last chamber in the series (Chamber III in Figure 1) becomes empty, it is filled with the buffer of next higher concentration of NaCl. Table 1 shows the buffer schedule for the gradient elution of skimmilk, casein, and whey proteins. Flow rates in the order of 1,000 ml/hour were obtained with a hydrostatic head of 100-120 cm. As in stepwise elution, the fractions were collected by a volumetric siphon and examined in a Beckman DU spectrophotometer at 280 mμ.

RESULTS

Determination of nitrogen recovery. Nitrogen recovery was determined by applying 50 ml. of dialyzed skimmilk on a DEAE column and washing it with five 20-ml. portions of starting buffer. The protein of skimmilk was eluted with 0.500 *M* NaCl buffer, and the effluent was collected in a 1-liter volumetric flask and made up to exactly 1,000 ml. The total nitrogen in the dialyzed skimmilk and on the aliquots of the effluent was determined by a semimicro-Kjeldahl method (16). Recovery was calculated at 94% or more. A further increase in

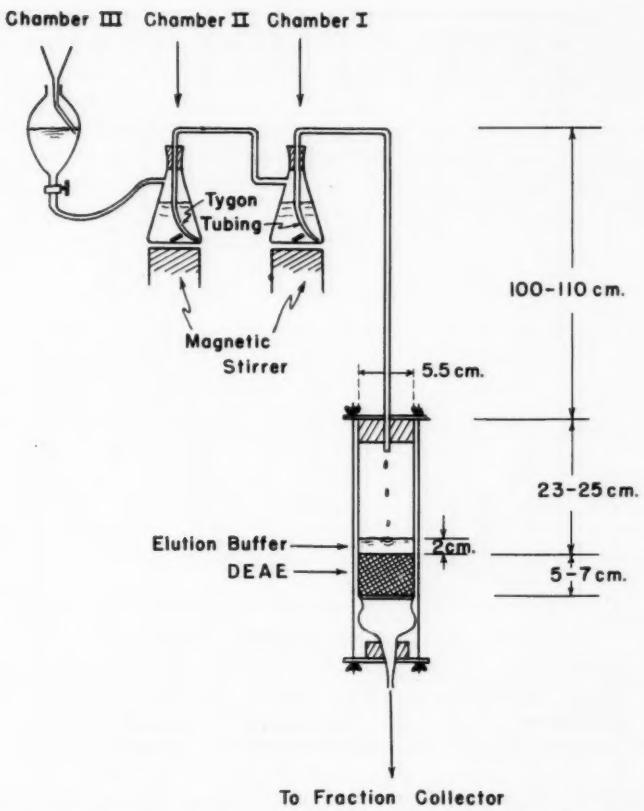

FIG. 1. Diagram of the apparatus used for gradient elution with two mixing chambers.

ionic strength up to 2 *M* of NaCl did not increase nitrogen recovery significantly, but in subsequent experiment the follow-up elution with NaOH gave an additional recovery of nitrogen.

Separation of proteins of skimmilk by stepwise elution. Fifty milliliters of dialyzed skimmilk containing 1.31 g. protein [6] was applied to DEAE column (7.0 by 5.5 cm.) and eluted with 15 different buffers, as shown in Table 1. The effluent diagram obtained is shown in Figure 2. Each buffer yielded a corresponding peak, forming 15 fractions, and these are named alphabetically and used in the other figures in this paper to indicate the relative position of the peaks without direct proof of identity. From the nitrogen recovery obtained, it is apparent that nearly all proteins of milk are eluted. The area estimated under the largest peak, *k*, comprises 15.9% of the total proteins of the skimmilk applied on the column. Since the content of α-casein as per cent of total protein

[6] Protein content calculated by multiplying total nitrogen by the factor 6.38.

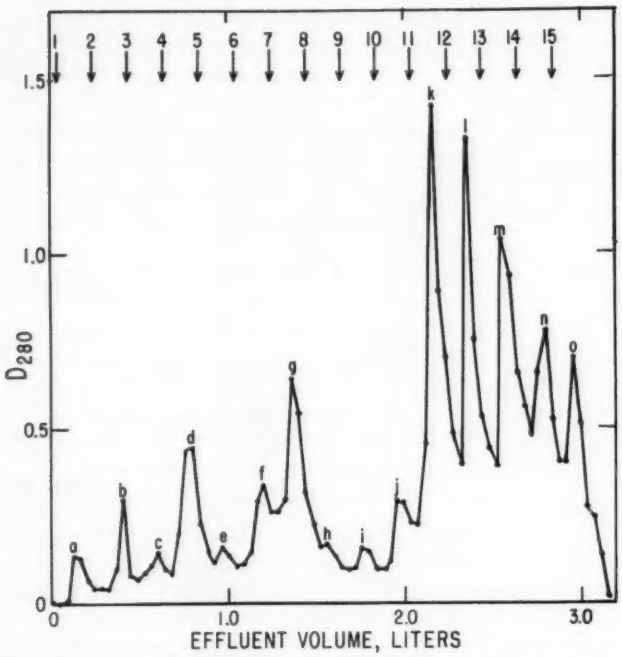

FIG. 2. Effluent diagram of proteins of skimmilk by stepwise elution; 50 ml. of dialyzed skimmilk (1.31 g. protein) was applied to DEAE column (7.0 by 5.5 cm.). Forty-milliliter fractions were collected at a flow rate of 800 ml/hour (3-6 p.s.i. N_2, room temperature). The number and vertical arrow show the point of change of the eluting buffer added to the column.

varies from 45 to 63% (8), the data indicate that α-casein is further fractionated by this method. The data suggest that β-casein is also a mixture of proteins, since the β-casein fraction varies from 19 to 28% of total milk proteins (8).

Position of casein and whey protein fractions on effluent diagram. In the study of milk protein by electrophoresis, preliminary separation of casein and whey protein fractions is essential to prevent their electrophoretic peaks from overlapping. To determine the position of casein and whey protein fractions in the effluent diagram obtained with skimmilk (Figure 2), purified isoelectric casein (1.46 g. protein) and acid whey (0.49 g. protein) were prepared from the same skimmilk and chromatographed separately, using the identical elution schedule used for the skimmilk. The results are in Figures 3 and 4.

Casein and whey proteins are clearly and distinctly separated. Whey proteins are eluted in the region of zero to 0.2 *M* NaCl concentration in the buffer. It will be noted that two fractions of whey proteins (Peaks a_1, a_2) are breakthrough fractions. They are not adsorbed on the column at the pH of buffer solution used.

When the concentration of NaCl in the buffer exceeds 0.20 *M*, casein starts to emerge and apparently most of the casein fractions are eluted before the

NaCl concentration in the buffer reaches 0.500 *M*. No whey proteins are eluted in this region, as is clearly seen in Figure 3. The data of Figures 3 and 4 show at least nine different protein components in the whey protein fraction, and seven in casein.

Comparison of Figure 2 with Figures 3 and 4 illustrates the reproducibility of both the position and relative amounts of the various protein components.

Separation of casein by gradient elution. The number and relative amounts of each protein component in the closely related mixture of proteins could be resolved more explicitly by gradient elution.

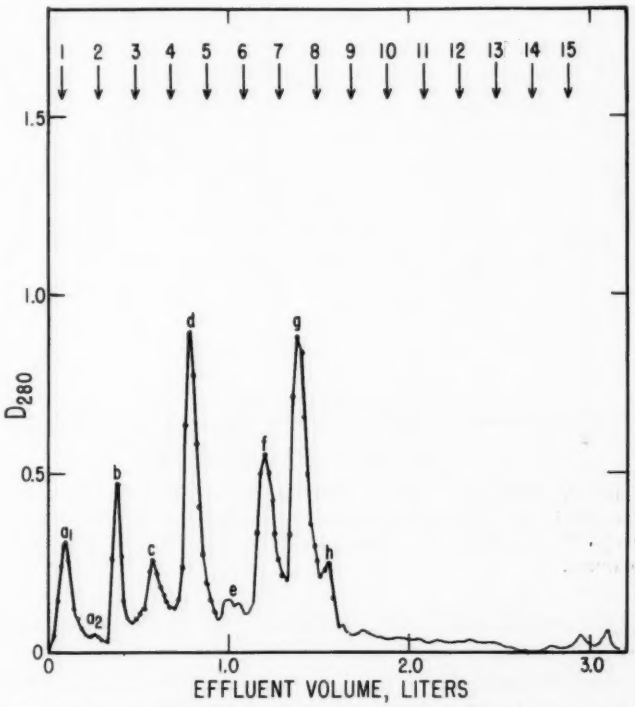

FIG. 3. Effluent diagram of whey proteins by stepwise elution; 75 ml. of dialyzed whey (0.486 g. protein) was applied to DEAE column (7.0 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 1,100 ml/hour (3-6 p.s.i. N_2, room temperature).

The same amount of the identical casein used in the stepwise elution experiment (Figure 4) was placed on the column and eluted with the buffer containing a gradual increase in concentration of NaCl. The elution schedule is shown in Table 1, and the results in Figure 5. The peaks of Figure 5 corresponding to those of Figure 4 can be identified approximately from the order of elution and the area under the peaks. It is seen that some of the casein peaks obtained by stepwise elution as employed in this study are still heterogeneous.

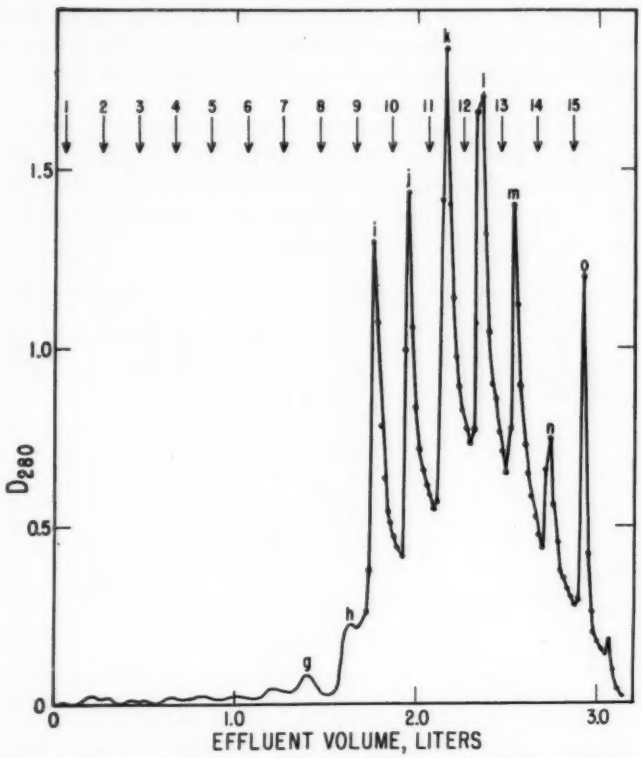

FIG. 4. Effluent diagram of casein by stepwise elution; 50 ml. of dialyzed preparation of purified isoelectric casein (1.46 g. protein) was applied to a DEAE column (6.4 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 1,000 ml/hour (3-6 p.s.i. N_2, room temperature).

Assuming that several small peaks, including Peak *h*, of the initial portion of the effluent are contaminants of whey proteins, the gradient elution of casein yielded at least nine, and possibly 11, components, depending on whether Peaks j_2, j_3, and k_1, k_2 represent two or four different fractions.

Separation of whey proteins by gradient elution. The whey used in this experiment was from milk from a different cow than that used in the experiment of Figures 2, 3, 4, and 5. Two mixing chambers were used for gradient elution of 150 ml. of dialyzed whey proteins. The results are in Figure 6. From the order of elution and relative amounts, peaks corresponding to the peaks of Figure 3 are identified.

Gradient elution did not give a greater number of components for whey protein than were obtained by a stepwise method. The Peak *g* of Figure 3 is not present in the effluent diagram of Figure 6. Its absence was understood after chromatographic identification of β-lactoglobulin.

Chromatographic identification of β-lactoglobulin. An unknown component in the mixture of closely related proteins can be identified if the purified known component is available and is chromatographed under exactly the same conditions as the mixture of the proteins in which it may be present. Purified β-lactoglobulin, which is commercially available,[2] was used as an example of chromatographic identification.

Dialyzed β-lactoglobulin was chromatographed by stepwise and gradient elutions (Figures 7 and 8) by the same procedures used in the experiments of Figures 3 and 6. It can be seen that the position of β-lactoglobulin in Figure 7 corresponds exactly to the positions of the Peaks *f* and *g* in Figure 3, and the position of β-lactoglobulin in Figure 8 corresponds to the peak of *f* in Figure 6. It is evident that Peaks *f* and *g* represent β-lactoglobulin fractions.

It has been established (1, 2) that β-lactoglobulin consists of two very similar proteins, called β-lactoglobulin A and β-lactoglobulin B. Some cows produce only A, some only B, and some both (1). Commercial β-lactoglobulin (Figures 7 and 8) gave two peaks, which would be expected if it were isolated

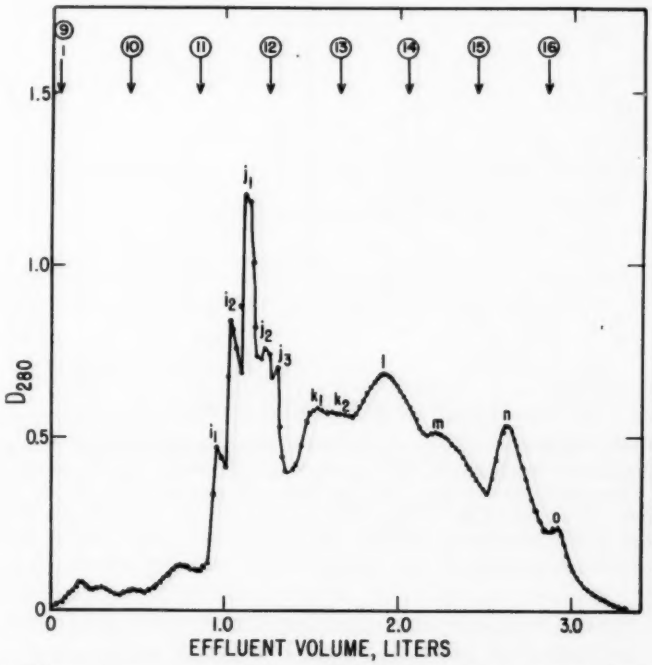

FIG. 5. Effluent diagram of casein by gradient elution with a mixing chamber; 50 ml. of dialyzed preparation of purified isoelectric casein (1.46 g. protein) was applied to a DEAE column (7.0 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 1,200 ml/hour (hydrostatic pressure 110 cm., room temperature). The number in a circle and the vertical arrow show the point of change of the eluting buffer added to Chamber II.

from a mixed milk. As stated before, the milk used in the experiment of Figure 6 was from a different cow from that used in Figure 3. Whey from the milk used in Figure 3 has both β-lactoglobulin in fractions (Peaks *f* and *g*). Whey of Figure 6 has only one fraction. Since electrophoretic mobility at pH 8.6 is slower for β-lactoglobulin B than for β-lactoglobulin A (2), it would be reasonable to assume, in view of close similarity of these fractions, that Peaks *f* and *g* in Figure 3 are, respectively, β-lactoglobulin B and β-lactoglobulin A, and that the single peak in Figure 6 is β-lactoglobulin B.

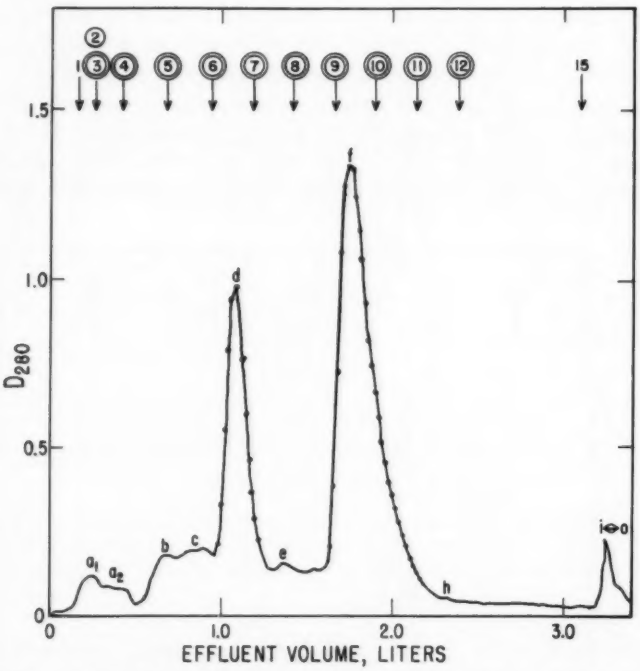

FIG. 6. Effluent diagram of whey proteins by gradient elution with two mixing chambers; 150 ml. of dialyzed whey proteins (0.693 g. proteins) was applied to DEAE column (8.0 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 1,020 ml/hour (hydrostatic pressure of 110 cm., room temperature). The number in a double circle is the number of the eluting buffer added to Chamber III. The number in a single circle is the number of the buffer in the second chamber. The Buffers 1 and 15 were added directly on a column.

In identifying components chromatographically it must be kept in mind that the components on the effluent diagram obtained by gradient elution will shift to the right from their position on the stepwise elution diagram. The extent of the shift is determined by the number of mixing chambers. In stepwise elution the number of the buffer shown on diagrams is the buffer with respect to NaCl concentration and pH with which the particular component is eluted.

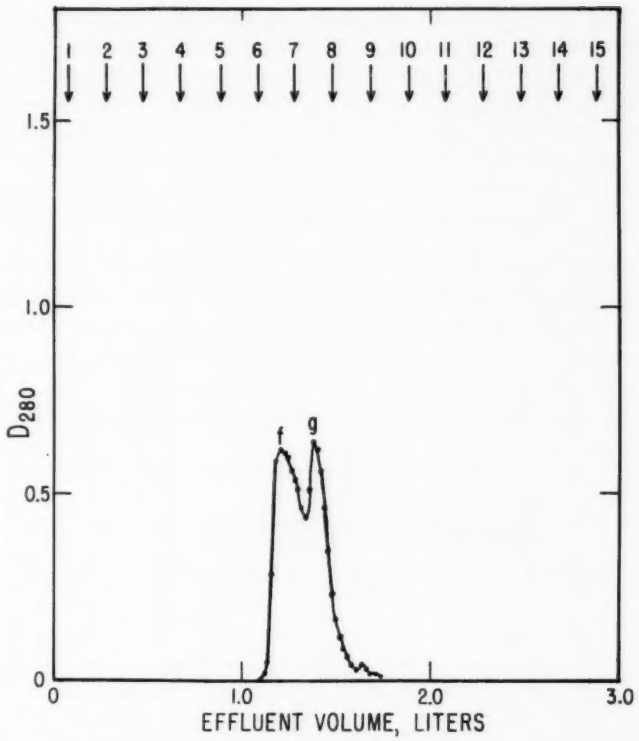

FIG. 7. Effluent diagram of β-lactoglobulin by stepwise elution; 150 ml. of dialyzed commercial β-lactoglobulin (0.295 g. protein) was applied to DEAE column (8.5 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 900 ml/hour (3-5 p.s.i. N_2, room temperature).

In gradient elution the number of the buffer shown on diagrams corresponds to the concentrations of NaCl in the buffer of the initial chamber (Chamber III in Figure 1). The buffer is mixed in the subsequent chambers. The effective concentration of NaCl in elution buffer remains the same in both cases.

Chromatographic identification of other milk protein components. In a progress of this study, Zittle (30) reported that κ-casein is not eluted from DEAE cellulose column with 1 *M* NaCl, but it is displaced by OH ions. Accordingly, in chromatographing α-lactalbumin, α-casein, β-casein, and κ-casein, the elution with NaCl was followed up by elution with NaOH, as indicated in Table 1. In all respects, the procedure was the same as in stepwise elution of previous experiments. For reference and comparison, a sample of dialyzed skimmilk that was different from that in Figure 2 and represented the mixed milk of three Holstein cows was chromatographed, and also eluted with NaOH. The data of these experiments are presented in Figures 9, 10, 11, and 12, and

on the basis of these data the peaks are identified as indicated in Figure 13.

The data indicate that preparation treatments of samples of α-casein, β-casein, and κ-casein did not change their position on chromatograms as judged by the position of respective peaks on chromatogram of dialyzed skimmilk. In the case of α-lactalbumin the position of the major peak, e_1 (Figure 9), which apparently corresponds to Peak d on the chromatogram of dialyzed skimmilk (Figure 13), has been shifted slightly to the right. Furthermore, the shoulder of the e_1 peak, Peak d on the α-lactalbumin chromatogram, is more pronounced, suggesting the presence of two molecular species in purified α-lactalbumin. The reason for this change is not clear at present. Klostergaard and Pasternak (11), on the basis of electrophoretic experiments, suggested that "when α-lactalbumin is dissolved in buffer solutions changes take place as a result of which the final solution contains the protein in more than one molecular form." Zittle and Della-Monica (31) and Zittle (29) also indicated the similar effects of lyophilizing and salts on α-lactalbumin. The α-lactalbumin (Figure 9) appears to be

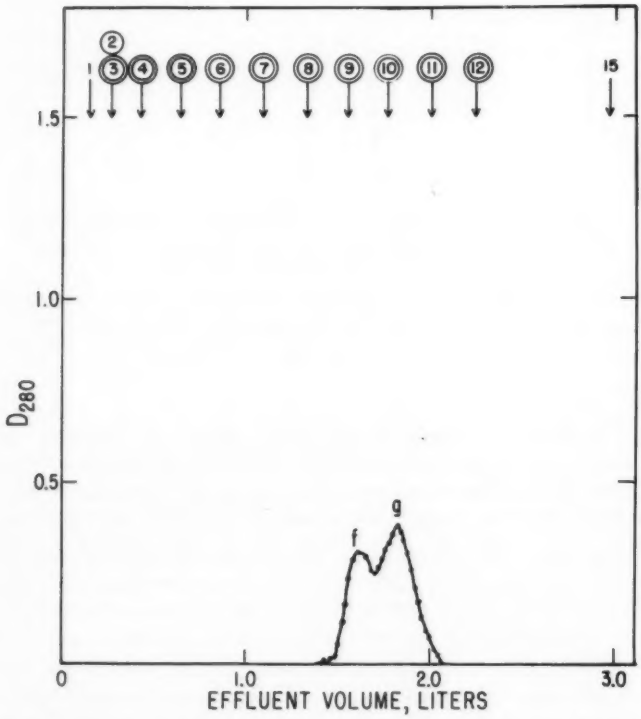

FIG. 8. Effluent diagram of β-lactoglobulin by gradient elution with two mixing chambers; 140 ml. of dialyzed commercial β-lactoglobulin (0.208 g. protein) was applied to DEAE column (8.0 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 1,100 ml/hour (hydrostatic pressure of 100 cm., room temperature).

quite pure, but even though it was recrystallized four times it still contained a minute amount of β-lactoglobulin (Peaks f and g) and a trace of unidentified component (Peak h). The significance of the presence of small peaks in the casein region (Peaks p, z_2, z_3, z_4), mainly in the region of elution with NaOH, is not understood at this time.

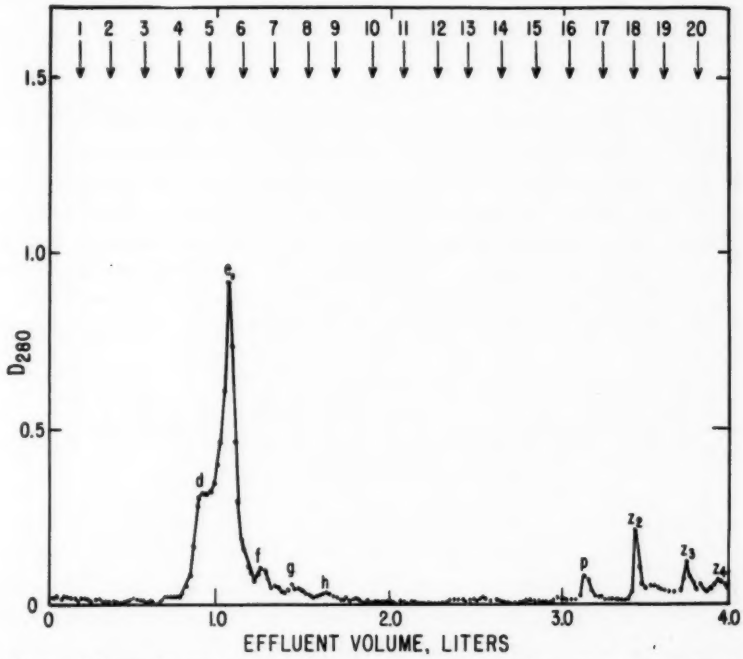

FIG. 9. Effluent diagram of α-lactalbumin by stepwise elution; 25 ml. of dialyzed α-lactalbumin (100 mg. protein) was applied to DEAE column (5.0 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 800 ml/hour (1-2 p.s.i. N_2, room temperature).

The chromatographic data on α-casein (Figure 10) show that it is entirely free of whey protein components and the casein Peaks i, j, and k. The Peaks j and k (Figure 11) were identified as β-casein. The Peaks l, m, n, o, p, z_1, and z_2 are components of α-casein. The Peaks z_3 and z_4 appear on chromatograms of whey proteins also (23) and, therefore, remain unidentified. The α-casein preparation which, as stated in the experimental part, showed a single electrophoretic peak, consists of at least seven chromatographic components. The data of Figure 11 show that β-casein preparation is extensively contaminated with whey components (the Peaks d, f, and g) and, interestingly enough, only with certain components of α-casein, including a significant amount of κ-casein (compare with Figure 12). The Peaks j and k seem to be distinctive for β-casein, not being present in α-casein (Figure 10). Kappa-casein preparation shows one

major peak, z_2, and minor peaks as contaminants with other components of α-casein. It is free of β-casein and whey proteins.

The Peaks *a* and *b* were identified as lactoglobulin fraction on the basis of chromatographing separately lactoglobulin and lactalbumin fractions. The fractions were prepared by the separation of lactoglobulin at 0.5 saturation with $(NH_4)_2SO_4$ and further purification of the fractions as described by Smith (18). The chromatogram of lactalbumin was entirely free of Peaks *a* and *b*, but the same peaks were the major peaks in the chromatogram of lactoglobulin.

DISCUSSION

The results indicate that anion exchange cellulose is very effective in separating milk proteins. The apparent advantages over conventional electrophoresis and other methods are high resolving power, and minimum denaturation, since fractionation of the proteins can be made directly from milk with no treatment other than dialysis and elution with phosphate buffer at relatively low levels of NaCl concentration. In addition, the method allows the isolation of a particular protein component in quantities sufficient for analytical or preparatory purposes. The comparison of corresponding peaks on the chro-

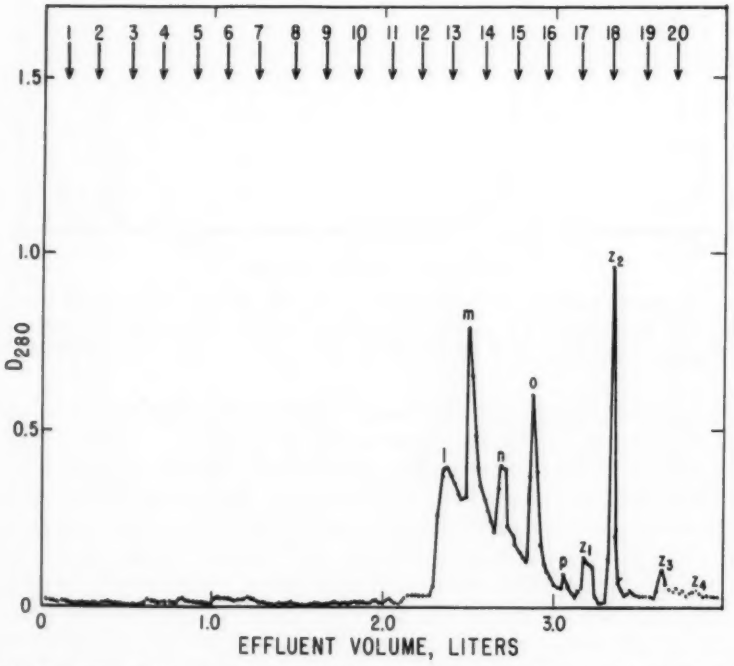

FIG. 10. Effluent diagram of α-casein by stepwise elution; 25 ml. of dialyzed α-casein (300 mg. protein) was applied to DEAE column (5.3 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 800 ml/hour (1-2 p.s.i. N_2, room temperature).

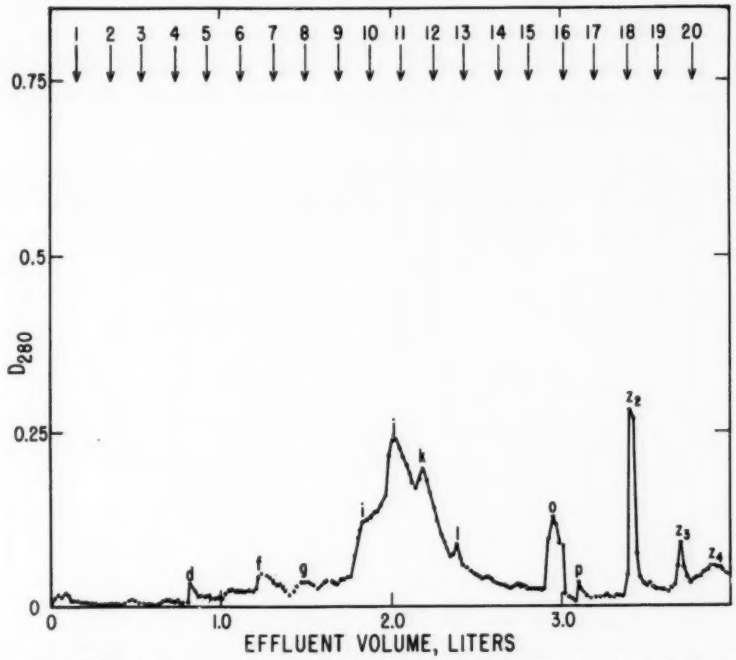

FIG. 11. Effluent diagram of β-casein by stepwise elution; 25 ml. of dialyzed β-casein (361 mg. protein) was applied to DEAE column (5.5 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 800 ml/hour (1-2 p.s.i. N_2, room temperature).

matograms of two different dialyzed skimmilks (Figures 2 and 13) and the chromatographic data on the purified preparation (Figures 9, 10, 11, and 12) demonstrate the reproducibility of the method and its value for identifying the protein components and determining the extent of contamination in a given preparation.

The disadvantage of anion exchange cellulose chromatography has been a time-consuming factor in elution. The column previously used (28) required 50 to 60 hr. for complete fractionation of milk proteins. In the present study it was found that introducing Jennings' device (9), and modifying the column to be shorter and wider, as suggested by Rhodes *et al.* (15), reduced the time for fractionation to 3-5 hr., for either stepwise or gradient elution. Shortening the column gave a much higher flow rate, giving more rapid separation with no significant decrease in resolving power in the case of milk proteins.

The present method employed salt gradient rather than pH gradient for elution. Thus, milk proteins can be fractionated at a pH essentially near that of milk, except for the κ-casein fraction, which apparently is more strongly adsorbed and is eluted with NaOH. Fractionation of proteins near the pH of milk is particularly advantageous for isolating and purifying enzymes such as milk lipases, which are quite sensitive to changes in pH (4).

Separation of the whey protein fraction and the casein fraction by DEAE cellulose chromatography is complete and clear-cut, without overlapping peaks. Whey protein components are eluted in the range of 0 to 0.2 *M* NaCl. Casein components do not begin to emerge from the column until the concentration of NaCl in the eluting buffer exceeds 0.2 *M*.

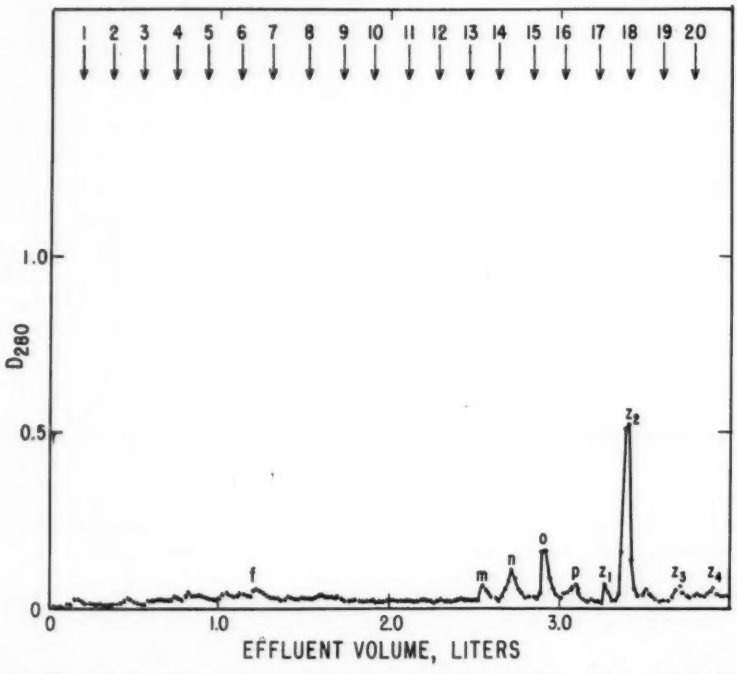

FIG. 12. Effluent diagram of kappa-casein by stepwise elution; 25 ml. of dialyzed κ-casein (100 mg. protein) was applied to DEAE column (5.0 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 800 ml/hour (1-2 p.s.i. N_2, room temperature).

The present status of the extent of heterogeneity of casein is rather confusing, especially with respect to the nomenclature of α-casein components. McMeekin *et al.* (13) recently showed that α-casein at pH 2.35 consists of at least three electrophoretic components (α_1, α_2, α_3). They found that α_1-casein itself has two components with different sedimentation constants. Thus, casein consists of at least four different fractions of α-casein besides β- and γ-caseins. It is not entirely clear whether the so-called κ- and m-caseins (24, 26, 27) and λ-casein (12) are different from or identical to the components of α-casein reported by McMeekin *et al.* (13). Using agar and starch gel electrophoresis, Wake and Baldwin reported nine electrophoretically separate components of casein (25). The present study clearly shows that α-casein is highly heterogeneous and contains more components than generally has been accepted.

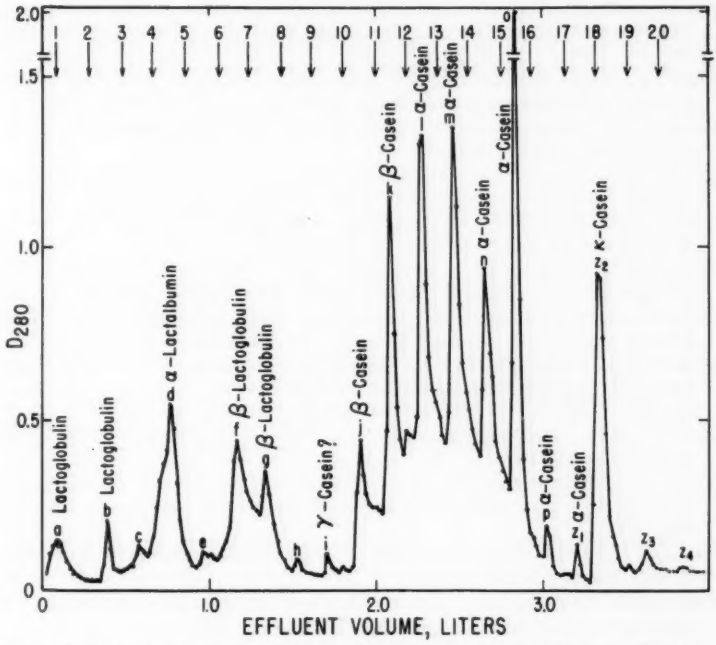

FIG. 13. Effluent diagram of proteins of skimmilk by stepwise elution; 50 ml. dialyzed skimmilk (1.49 g. protein) was applied to DEAE column (5.5 by 5.5 cm.). Twenty-milliliter fractions were collected at a flow rate of 800 ml/hour (1-2 p.s.i. N_2, room temperature).

ACKNOWLEDGMENT

The authors are grateful to Dr. R. Jenness, University of Minnesota, for the samples of α-lactalbumin, α-casein, β-casein, and to Dr. Zittle of Eastern Research and Development Division, USDA, for the sample of κ-casein.

Dr. W. G. Jennings gave valuable suggestions for improving the efficiency of the DEAE column.

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QUANTITATIVE FATTY ACID ANALYSIS OF MILK FAT BY GAS-LIQUID CHROMATOGRAPHY

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SUMMARY

A procedure was developed for quantitative analysis of the fatty acids of milk fat. Methyl esters of the acids were prepared by methanolysis, extracted with ethyl chloride, and separated by gas-liquid chromatography (GLC), with diethylene glycol succinate as liquid phase. Known mixtures of methyl esters were used in determining factors for correction of peak areas for losses from evaporation of short carbon chain esters and/or variations in relative detector response among the esters under the conditions of the actual analysis. Replicate samples of milk fat esters were analyzed and compared with data obtained by spectrophotometry and with results of others determined by ester distillation and GLC techniques. All the major and many of the known minor fatty acids of milk fat were determined. Advantages and limitations of the method are discussed.

Previous ester fractionation techniques for analyzing the fatty acid composition of milk fat have many limitations (11, 13). In general, they demand a high degree of skill, are laborious and time-consuming, require large samples, and do not yield precise values for either the lower-molecular-weight or the unsaturated fatty acids. Therefore, these methods have not been used routinely for the examination of milk fat and there is urgent need for a simpler and more precise method. Obviously, such a technique would have many applications in research and dairy technology.

Gas-liquid chromatography (GLC) has made possible the rapid and convenient separation and isolation of minute quantities of a wide variety of complex mixtures, including mixtures of fatty acids and their esters (3, 4, 8, 16, 17). Although James and Martin (15) used GLC as early as 1956 in analyzing the saturated fatty acids of goat milk fat, the technique has not been widely applied in total analysis of the fatty acids of bovine milk fat. The unusually wide variety of the acids in this fat presents problems. For example, it is difficult to prepare a mixture of esters that is representative of the original fatty acid composition because the lower-molecular-weight acids and their methyl esters are relatively volatile and may be lost during solvent removal prior to GLC separation. Furthermore, the great differences in molecular weights of the esters make it difficult to obtain optimum resolution of both short- and long-chain esters without resorting to the use of more than one column temperature, temperature programming, or two separate columns.

A number of investigators have employed GLC to analyze the composition of milk fat fractions that contained mainly either volatile or nonvolatile fatty acids. Hawke (9) separated the steam-volatile acids and the methyl esters of the nonvolatile acids. Patton and Keeney (19) and, more recently, Thompson, Brunner, and Stine (27) analyzed high-melting glyceride fractions from milk

Received for publication September 27, 1960.

fat. Haab, Smith, and Jack (5) employed GLC in studying the composition of triglyceride fractions separated by countercurrent distribution. Hansen, Shorland, and Cooke (7) used the technique to confirm the presence in milk fat of very small amounts of the normal odd-numbered saturated C_{19}, C_{21}, and C_{23} acids and the normal even-numbered series from C_{20} through C_{26}. Patton, Evans, and McCarthy (18) reported on the C_8 through C_{18} fatty acids of milk fat and fractions produced by pancreatic lipase. Samuels *et al.* (21) recently gave a more complete fatty acid analysis of a milk fat by GLC.

This paper presents a comparatively simple GLC procedure for the quantitative analysis of all the major and many of the minor fatty acids of milk fat. Analyses by this method and by spectrophotometry are compared with data reported by others determined by ester fractionation and GLC.

EXPERIMENTAL PROCEDURE

Preparation of synthetic mixtures of methyl esters. All synthetic mixtures were prepared by weighing the required amounts of highly purified methyl esters of saturated and unsaturated fatty acids.[1] Methyl butyrate was always weighed last, and evaporation was minimized by keeping the sample tubes tightly stoppered. The purity of each ester was previously checked by GLC and, where necessary, minor corrections were made in the calculated weight per cent composition of each mixture.

Preparation of milk fat. Milk fat samples were obtained by churning cream, melting the butter granules, and filtering the clear fat at 50° C. through Whatman No. 12 filter paper. Milk fat was hydrogenated with a Parr Hydrogenation Apparatus operated 2 hr. at 27° C. under 20 p.s.i. of hydrogen. The catalyst was 5% platinum on carbon powder,[2] and pentane was employed as solvent.

Determination of polyunsaturated fatty acids. These were determined by the macro-ultraviolet spectrophotometric technique described elsewhere (25). Iodine values were obtained by the Hanus procedure (25).

Preparation of milk fat methyl esters. The following methanolysis procedure was used to prepare methyl esters of the fatty acids:

Materials. Ethyl chloride—U.S.P.

Methanol—reagent grade, refluxed and redistilled over zinc dust and potassium hydroxide (KOH).

0.1 *N* KOH in methanol—about 0.56 g. KOH pellets in 100 ml. of methanol.

Bromcresol purple solution—0.04% aqueous.

0.1 *N* hydrochloric acid (HCl).

Test tubes—16 by 125 mm., with air-tight screw caps lined with polyethylene.

Centrifuge tubes—13 ml., conical with air-tight screw caps.

Centrifuge—Servall, Model SS1A.

Siphon and cap—as in Figure 1.

[1] California Corporation for Biochemical Research, Los Angeles 63, Calif.

[2] Baker and Co., Inc., Newark 5, New Jersey.

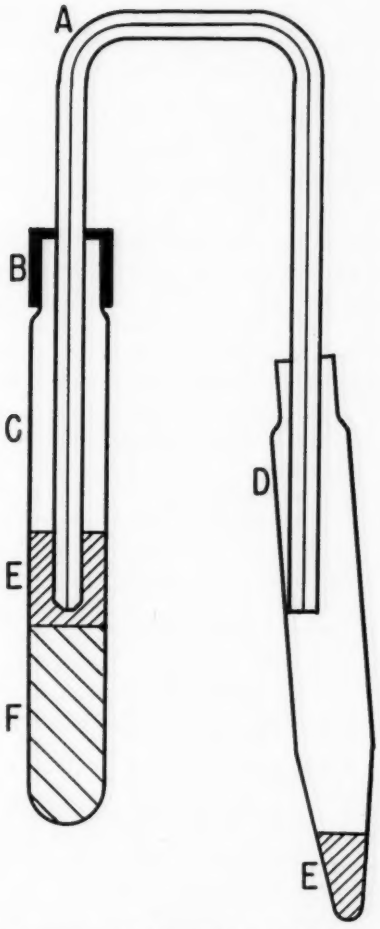

FIG. 1. Apparatus for preparation of fatty acid methyl esters of milk fat. A—capillary tubing siphon that can be lowered through hole in Cap B; B—polyethylene-lined screw cap; C—screw cap test tube, 16 by 125 mm.; D—screw cap conical centrifuge tube, 13 ml.; E—ethyl chloride layer; F—aqueous phase.

Method. About 0.2 g. of melted milk fat (measured with a dropper) was placed at the bottom of a test tube, 2 ml. of ethyl chloride was added, and the tube was swirled to dissolve the fat. After addition of 2 ml. of 0.1 *N* methanolic KOH, the capped tube was heated 1 hr. in a water bath at 50° C. Then the tube was cooled in ice water; one drop of bromcresol purple and sufficient 0.1 *N* HCl to lower the pH below 5.2 were added, followed by 4 ml. of water. The methyl esters were extracted with 2 ml. of ethyl chloride by inverting the capped tube five times. After centrifuging the tube 3 min. at about 2,500 r.p.m., the cap

was removed and replaced with the siphon and its cap, care being taken to keep the end of the siphon well above the ethyl chloride. The other leg of the siphon was placed in a chilled centrifuge tube. Then the siphon was lowered rapidly about half-way into the ethyl chloride layer and, as this layer was siphoned over into the centrifuge tube (Figure 1), the siphon was slowly lowered until about one-tenth of the layer was left. At this stage, the siphon and special cap were removed. This extraction procedure was repeated twice more, using 2 ml. of ethyl chloride each time.

Most of the ethyl chloride was evaporated at room temperature in about 2 hr. by removing the cap from the centrifuge tube. The methyl ester mixture was examined promptly by GLC.

Gas chromatography. The instrument used was an Aerograph Model A-90-C[3] fitted with a four-filament thermal conductivity cell. Filament amperage was 230 ma. Two 6-ft. columns of ¼-in. O.D. stainless steel were employed. Column packing was 20% diethyleneglycol succinate (DEGS) on Chromosorb 35/80 mesh, but different lots were used for the two columns. Helium flow was controlled by a precision reducing valve and measured by a soap-bubble flow meter at the column outlet. Chromatograms were recorded with a 1-mv. full-scale recorder (Honeywell-Brown Electronik) equipped with a switch to change chart speed from 200 to 20 inches/hour. The column preheater and outlet tube were respectively 60 and 50° C. above the temperature at which the column was operated.

One-milliliter samples were injected with a 10.0-μl. Hamilton micro-syringe. Column temperatures were within the range 196-199° C., and helium flow rate was 60 ml/min. Separations of esters of saturated and unsaturated acids ranging from C-4 through C-18 were complete in less than 30 min.

RESULTS AND DISCUSSION

Analyses of synthetic mixtures. During development of the method, synthetic mixtures of methyl esters of about the major fatty acid composition of milk fat were used extensively as an aid in choosing the experimental parameters of operation, i.e., sample size, temperature, flow rate, etc., for identification and estimation of fatty acid esters.

In a representative experiment, a mixture of saturated and unsaturated methyl esters was prepared, and eight 1.0-μl. aliquots were examined with the gas chromatograph. Figure 2 shows the chromatogram of one of the aliquots. The peaks are well separated and tall and narrow. The area under each peak was obtained by multiplying peak height by peak width at half height (16). Areas under the stearate and oleate peaks were calculated by the method of approximating triangles (20). To compensate for minor variations in sample size, an internal normalization procedure was followed. The area of each peak was expressed as a percentage of the total peak area for all components. The known composition of the mixture is compared with the average observed area per cent for each component (Table 1).

[3] Wilkens Instrument and Research, Inc., Walnut Creek, California.

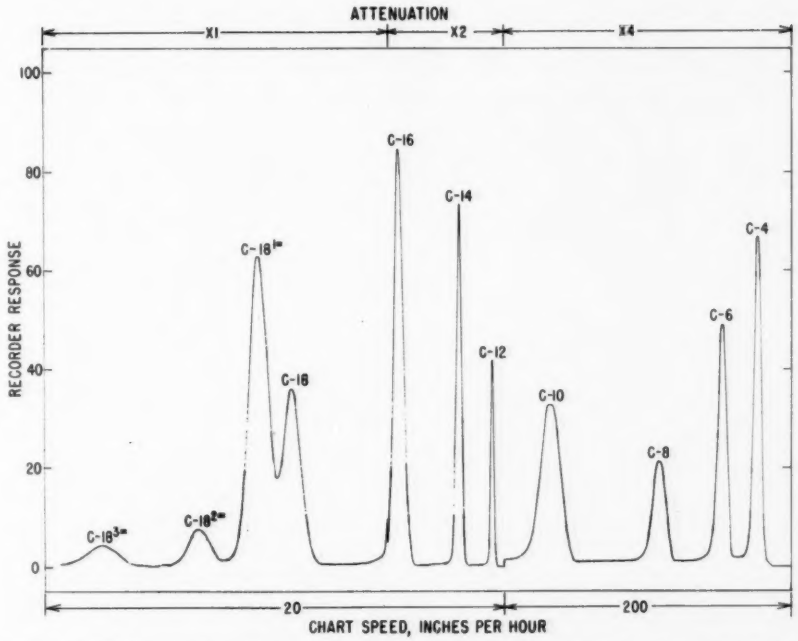

FIG. 2. GLC separation of components of known mixture of methyl esters of saturated and unsaturated acids from C-4 to C-18$^{3=}$ on Column No. 1 at 199° C.

These results show that the area per cent for each ester checks more closely with weight per cent than mole per cent, but that in either case correction factors should be applied in quantitative work. The standard deviations indicate excellent precision and reproducibility of the observed peak area per cent data.

To study the relation between sample concentration and peak area, a series

TABLE 1

Analysis of synthetic mixture of fatty acid methyl esters by GLC [a]

Component	Shorthand designation	Known		Observed [b]	
		Weight *(%)*	Mole *(%)*	Area *(%)*	S.D.
Butyrate	C-4	3.01	7.25	3.43	0.076
Caproate	C-6	2.76	5.25	3.14	0.051
Caprylate	C-8	1.77	2.75	1.91	0.040
Caprate	C-10	4.32	5.75	4.85	0.191
Laurate	C-12	4.45	5.00	4.87	0.218
Myristate	C-14	13.93	14.00	14.28	0.224
Palmitate	C-16	29.59	26.75	28.73	0.399
Stearate	C-18	12.29	10.00	12.24	0.177
Oleate	C-18$^{1=}$	22.31	18.50	21.50	0.237
Linoleate	C-18$^{2=}$	3.03	2.50	2.84	0.248
Linolenate	C-18$^{3=}$	2.55	2.25	2.22	0.132

[a] Column No. 1 at 199° C.
[b] Average of eight separations.

was prepared of five standard mixtures containing five levels of each of the C-4, C-6, C-12, C-16, C-18, and C-18[1=] methyl esters. A 1.0-μl. portion of each mixture was run in the gas chromatograph, and the observed area per cent for each peak was calculated without internal normalization. Figure 3 shows the data for C-4, C-6, C-16, C-18, and C-18[1=]. There was a linear relation between detector response (peak area per cent) and concentration of each ester over a tenfold concentration range. On the basis of these results, it is reasonable to assume that there is a linear relation between peak area and concentration for the types of fatty acids and for the concentrations of each likely to be found in milk fat.

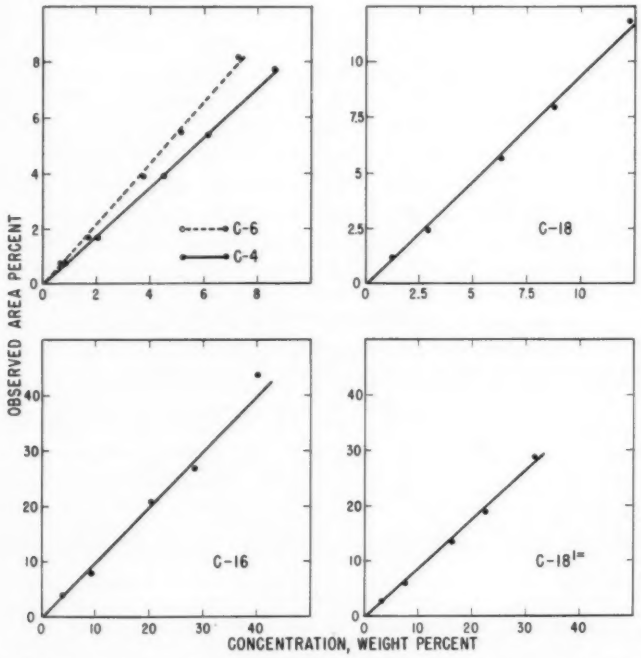

FIG. 3. Relation between observed peak area and component concentration during GLC separation of known mixtures of methyl esters of saturated and unsaturated fatty acids.

During the preparation of methyl esters from milk fat, it is necessary to evaporate most of the ethyl chloride used in the extraction step. To check on losses of the more volatile methyl esters during evaporation of the ethyl chloride, a synthetic mixture of methyl esters was made up and then six 0.2-g. portions were weighed into centrifuge tubes. Seven milliliters of ethyl chloride was added to each aliquot and allowed to evaporate at room temperature (25° C.). One microliter of each sample was then examined by GLC, and the area per cent calculated for each peak. Table 2 summarizes the results and

TABLE 2

Determination of correction factors to be applied to analysis of milk fat methyl esters by GLC[a]

Mixture component	Known weight (%)	Observed [b]		Recovery weight (%)	Area correction factor
		Area (%)	S.D.		
C-4	3.16	2.94	0.117	93	1.07
C-6	2.71	3.21	0.104	118	0.84
C-8	1.74	2.03	0.046	117	0.86
C-10	4.01	4.61	0.256	115	0.87
C-12	4.32	5.49	0.124	127	0.79
C-14	14.09	15.36	0.327	109	0.92
C-16	31.41	30.80	0.607	98	1.02
C-18	11.57	10.74	0.272	93	1.08
$C\text{-}18^{1=}$	22.89	21.23	0.258	93	1.08
$C\text{-}18^{2=}$	2.46	2.23	0.091	91	1.10
$C\text{-}18^{3=}$	1.62	1.39	0.123	86	1.17

[a] Column No. 2 at 198° C.
[b] Average of six GLC separations of synthetic mixture after evaporation of ethyl chloride.

lists the factors to correct for losses from evaporation and/or variations in area response per unit weight among these methyl esters. Since the composition of the synthetic mixture and the conditions of ethyl chloride evaporation were comparable, these factors were employed in the quantitative analysis of mixtures of milk fat methyl esters with Column No. 2. The standard deviations for the C-4 and C-6 area per cent data were higher than in Table 1, as might be expected, but they still indicated satisfactory precision.

Reproducibility of GLC analyses of milk fat. To study the variability of results in quantitative analysis of the fatty acid composition of milk fat by GLC, replicate samples were analyzed. In a typical experiment with Column No. 1, eight aliquots of the same fat (M-106) were taken through the methyl ester preparation and examination by GLC. The gas-liquid chromatograms were practically identical, and one of them is shown in Figure 4. Assignment of the major peak identities was made by determination of the corrected (1) retention volumes ($V_R°$) relative to methyl myristate, and comparison with those for the components of a synthetic mixture run under the same GLC conditions. Minor peaks were identified, as described later. Under the conditions used, methyl arachidonate and other minor components with carbon chains longer than C-18 were not resolved.

In the calculations, it was assumed that the total area under a chromatogram represented all the sample components. The normalized observed area per cent data were corrected for evaporation losses, etc. by the use of factors obtained as described above. In the case of the components that occurred in minor amounts for which factors were not available, the correction factor for the saturated ester with the same carbon chain length was employed. The calculated weight per cent of each ester was then converted to fatty acid weight per cent and mole per cent (Table 3). Variability is shown in terms of standard deviation. The C-4 data show the greatest variability, but further experience in our laboratory has demonstrated that better standardization of the ethyl chloride evaporation step reduces this variability markedly. Standard deviations of the C-6 through

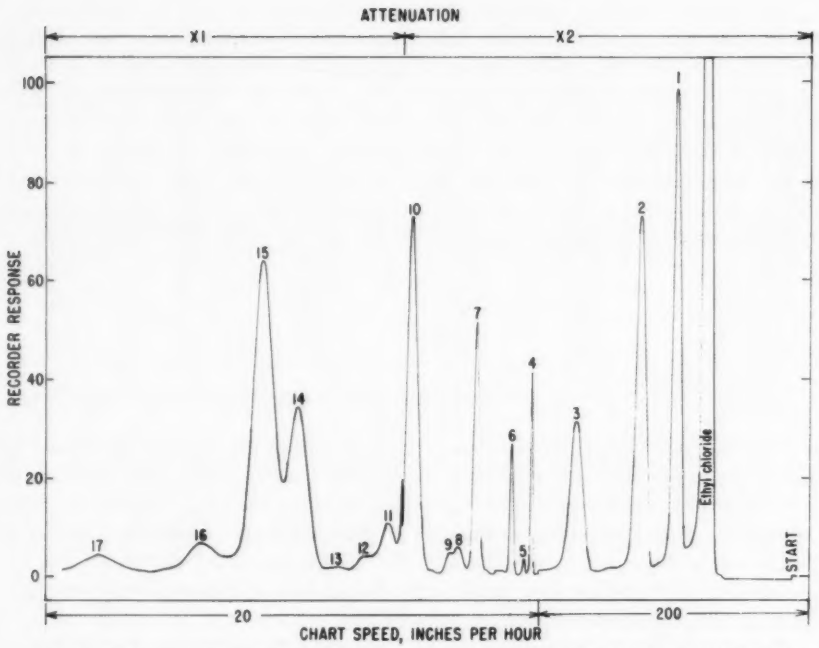

FIG. 4. GLC separation of fatty acid methyl esters of Milk Fat M-106 on Column No. 1 at 196° C. Peaks in order of appearance:
(1) C-4, (2) C-6, (3) C-8, (4) C-10, (5) C-10[1=], (6) C-12, (7) C-14, (8) C-14[1=], (9) C-15, (10) C-16, (11) C-16[1=], (12) C-17, (13) probably C-18[1∞], (14) C-18, (15) C-18[1=], (16) C-18[2=], (17) C-18[3=].

TABLE 3

Analysis of fatty acid composition of milk fat M-106 by GLC [a]

Peak	Relative retention volume [b] Unknown	Known	Component	Methyl ester Observed area (%)	Calculated wt. (%)	Fatty acid [c] Wt. (%)	S.D.	Mole (%)	S.D.
1	0.130	0.130	C-4	2.80	3.78	3.46	0.267	9.14	0.705
2	0.173	0.173	C-6	2.49	2.34	2.22	0.062	4.44	0.123
3	0.247	0.249	C-8	1.44	1.32	1.27	0.018	2.05	0.028
4	0.362	0.366	C-10	3.22	2.77	2.71	0.087	3.65	0.117
5	0.456		C-10[1=]	0.31	0.27	0.27	0.021	0.37	0.029
6	0.592	0.593	C-12	3.34	3.13	3.10	0.141	3.60	0.164
7	1.00	1.00	C-14	10.66	10.31	10.30	0.212	10.48	0.215
8	1.22		C-14[1=]	2.12	2.05	2.05	0.093	2.12	0.096
9	1.31		C-15	1.00	0.97	0.98	0.102	0.88	0.092
10	1.75	1.74	C-16	26.85	28.04	28.19	0.406	25.55	0.368
11	2.01		C-16[1=]	2.87	3.00	3.01	0.203	2.74	0.185
12	2.27		C-17	0.75	0.79	0.80	0.182	0.72	0.164
13	2.62		C-18[1∞]	0.17	0.18	0.18	0.066	0.14	0.051
14	3.05	3.01	C-18	12.78	10.59	10.70	0.150	8.74	0.122
15	3.46	3.42	C-18[1=]	24.02	24.85	25.10	0.389	20.66	0.320
16	4.15	4.12	C-18[2=]	3.28	3.46	3.59	0.293	2.88	0.241
17	5.33	5.27	C-18[3=]	1.91	2.17	2.19	0.257	1.84	0.216

[a] Column No. 1 at 196-198° C.
[b] Corrected retention volume (V_R°) relative to that of methyl myristate.
[c] Average of eight GLC separations.

C-18 saturated acids and of the C-14$^{1=}$ through C-18$^{1=}$ monoethenoid acids are appreciably less than those reported by Hansen (6) in a study of the accuracy of ester fractionation analysis of milk fat. Furthermore, Hansen's statistical analysis was based on only three analyses, whereas the data in Table 3 represent eight analyses.

The efficiency of a particular DEGS column gradually decreases with use, as evidenced by less complete resolution of the stearate and oleate peaks. Nevertheless, Brace (2) from studies of the quantitative evaluation of overlapping peaks, concluded that "resolution must be very poor indeed in order to interfere seriously with quantitative results by gas chromatography." Figure 5-A shows improved resolution of the minor peaks between C-12 and C-18 that was obtained with Column No. 2 prepared with a different lot of DEGS. At least 22 peaks are evident. Milk fat samples from several different cows have con-

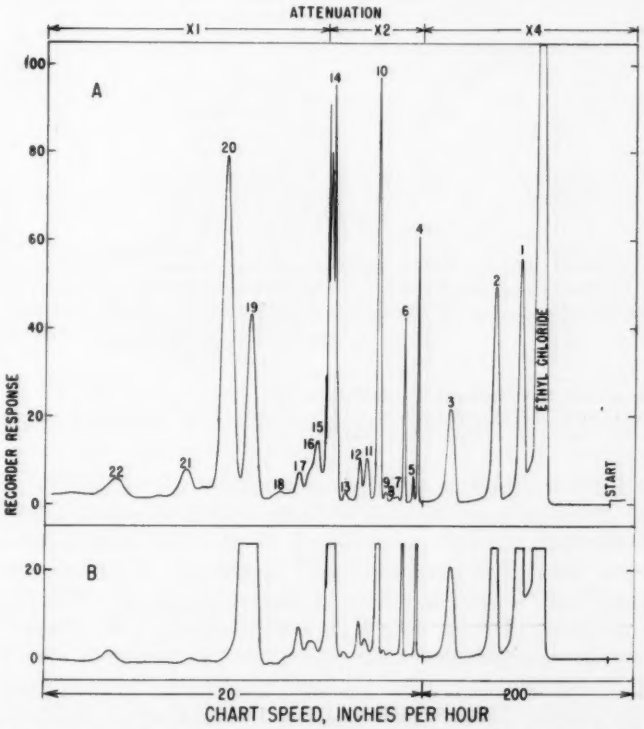

FIG. 5. GLC separation of fatty acid methyl esters of normal (5-A) and hydrogenated (5-B) milk fat on Column No. 2 at 198° C. Peaks in order of appearance: (1) C-4, (2) C-6, (3) C-8, (4) C-10, (5) C-10$^{1=}$, (6) C-12, (7) C-12$^{1=}$, (8) C-13, (9) probably C-14$^{1=o}$, (10) C-14, (11) C-14$^{1=}$, (12) C-15, (13) probably C-16$^{1=o}$, (14) C-16, (15) C-16$^{1=}$, (16) probably C-17$^{1=o}$, (17) C-17, (18) probably C-18$^{1=o}$, (19) C-18, (20) C-18$^{1=}$, (21) C-18$^{2=}$, (22) C-18$^{3=}$. Peak 14 was attenuated × 4.

sistently shown this same characteristic qualitative pattern when analyzed with this lot of DEGS.

From relative retention volumes obtained for a synthetic mixture of methyl esters, Peaks 1, 2, 3, 4, 6, 10, 14, and 19 were identified as the normal even-numbered saturated homologous series from C-4 through C-18. Similarly, Peaks 20, 21, and 22 were found to have respective retention volumes corresponding to C-18$^{1=}$, C-18$^{2=}$, and C-18$^{3=}$.

Identification of minor component fatty acids. Retention volumes of Peaks 6 through 19 were checked with data for known esters obtained by Hawke, Hansen, and Shorland (10) under comparable conditions (Table 4). The two sets of results show good agreement, and Peaks 8, 12, and 17 were tentatively identified as esters of C-13, C-15, and C-17 fatty acids. Peaks 9, 13, 16, and 18

TABLE 4

Retention volumes of methyl esters of C-12 through C-18 fatty acids of milk fat relative to methyl myristate

Peak	Relative retention volume		Methyl ester	Shorthand designation
	Unknown[a]	Known[b]		
6	0.58	0.57	*n*-Dodecanoate	C-12
7[c]	0.69		*cis*-Δ^9-Dodecenoate	C-12$^{1=}$
8	0.77	0.73	*n*-Tridecanoate	C-13
9	0.89	0.88	12-Methyltridecanoate	C-14$^{i\infty}$
10	1.00	1.00	*n*-Tetradecanoate	C-14
11[c]	1.20		*cis*-Δ^9-Tetradecenoate	C-14$^{1=}$
12	1.31	1.31	*n*-Pentadecanoate	C-15
13	1.54	1.56	14-Methylpentadecanoate	C-16$^{i\infty}$
14	1.81	1.77	*n*-Hexadecanoate	C-16
15[c]	2.00	2.08	*cis*-Δ^9-Hexadecenoate	C-16$^{1=}$
16	2.13	2.08	15-Methylhexadecanoate	C-17$^{i\infty}$
17	2.29	2.29	*n*-Heptadecanoate	C-17
18	2.60	2.78	16-Methylheptadecanoate	C-18$^{i\infty}$
19	3.08	3.09	*n*-Octadecanoate	C-18

[a] Column No. 2, 6 ft., liquid phase DEGS, at 198° C.
[b] Data of Hawke, Hansen, and Shorland (10). Column 8 ft., liquid phase DEGS, at 200° C.
[c] Peak not present in hydrogenated milk fat.

were tentatively identified as esters of *iso* branched-chain C-14, C-16, C-17, and C-18 acids. The very small amounts of methyl esters of *anteiso* branched-chain acids probably present were not resolved by the GLC conditions employed. The retention volume of Peak 15 closely approximated the value for *cis*-Δ^9-hexadecenoate and, since monoethenoid esters immediately follow saturated esters of the same molecular weight on DEGS columns (17), Peaks 7 and 11 were tentatively considered as C-12$^{1=}$ and C-14$^{1=}$, respectively.

Evidence supporting the above tentative assignments of peak identities was obtained by plotting carbon chain numbers for each of the three homologous series against the logarithms of the relative retention volumes (Figure 6), and by analyzing a hydrogenated milk fat under comparable GLC conditions (Figure 5-B). Figure 5-B shows that Peaks 8, 12, and 17 were not eliminated by hydrogenation and, therefore, were saturated. Their retention volume logarithms fell into the expected places for saturated C-13, C-15, and C-17 esters in Figure

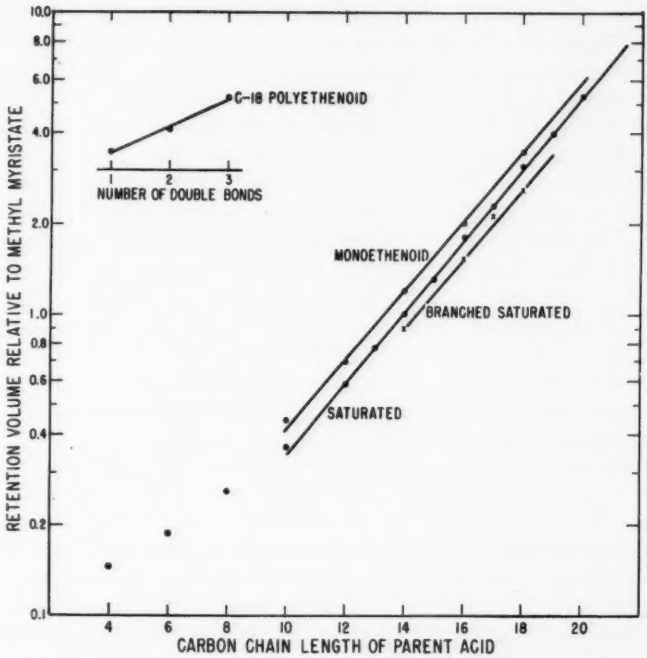

FIG. 6. Relation between $\log_{10}$ of the retention volume (relative to methyl myristate) and (a) the number of double bonds for the C-18 polyethenoid esters and (b) the number of carbon atoms in the parent acid for the methyl esters of straight-chain saturated, branched, and monoethenoid acids prepared from milk fat.

6. Peaks 9, 13, 16, and 18 were also not lost on hydrogenation (Figure 5-B), and fell along a straight line parallel to and immediately below the saturated homologous series (Figure 6). Peaks 5, 7, 11, 15, and 20 were practically eliminated by hydrogenation (Figure 5-B) and, therefore, were unsaturated. The retention volume logarithms for Peaks 5, 7, and 11 fell along the straight line passing through the points for C-$16^{1=}$ and C-$18^{1=}$ and running parallel to the line for the saturated series (Figure 6). Furthermore, the relative positions of the lines for the three homologous series agree with those shown by Hawke, Hansen, and Shorland. Although the above evidence for the identification of odd-numbered, *iso* branched-chain, and monoethenoid esters is strongly indicative, especially when considered in the light of the known occurrence of their parent fatty acids in milk fat (23), it can not be regarded as conclusive. Actual isolation and a chemical degradation study of these components were considered to be beyond the scope of the investigation.

Methyl arachidate (C-20) and methyl arachidonate (C-$20^{4=}$) were not detected with the 1.0-μl. sample routinely employed, but were resolved as comparatively flat peaks with 5.0-μl. samples. They were identified by running

known esters under the same GLC conditions. A representative milk fat (M-106) contained 0.5% arachidic and 0.3% arachidonic acid. The logarithms of the relative retention volumes of Peaks 21 and 22 in Figure 5-B fell into the expected places for C-19 and C-20 saturated esters (Figure 6). This indicates the presence of trace amounts of saturated and/or unsaturated C-19 constituents in addition to the C-20 components.

Figure 6 also shows that the logarithms of the retention volumes of Peaks 20, 21, and 22 are linear with respect to the number of double bonds in the molecule. The C-4, C-6, and C-8 members of the saturated homologous series deviate from linearity at the comparatively high column temperature used, but they can be readily identified by direct calibration with known esters.

The relative retention volumes of the C-8 through C-18[3=] components of milk fat obtained with Columns 1 and 2 (Tables 3 and 4) agree within $\pm 2\%$ of the average value for each. Therefore, new columns can be prepared and used for routine analyses without calibration, provided they are discarded promptly when they begin to lose separation efficiency.

Polyunsaturated fatty acid composition as determined by spectrophotometry and GLC. Spectrophotometric methods have been used to determine polyunsaturated fatty acids in a wide variety of lipids, including milk fat (13, 22, 25). In the present investigation, numerous milk fat samples were analyzed by both spectrophotometry and GLC. Data for two samples, representative of these analyses, are presented in Table 5. There is reasonably good agreement between the two methods in amounts of total saturated and monoethenoid constituents. Even so, only the GLC technique can determine qualitatively and quantitatively the individual saturated and mono-unsaturated acids. Under the con-

TABLE 5

Polyunsaturated fatty acid composition of milk fat as determined by spectrophotometry and GLC

	Weight per cent of fatty acids			
	Milk fat D-20		Milk fat H-14	
	Iodine value 27.4		Iodine value 27.7	
	Spectro	GLC	Spectro	GLC
Conjugated				
Diene	0.48		0.46	
Triene	0.02		0.02	
Tetraene	0.002		0.004	
Nonconjugated				
Monoene	20.67[a]	22.96	22.39[a]	24.59
Diene	1.98	2.28	1.71	2.11
Triene	0.74	1.40	0.68	1.16
Tetraene	0.33		0.27	
Pentaene	0.30		0.21	
Total polyunsaturated	3.87	3.68	3.35	3.27
Total saturated	75.48[b]	73.36	74.26[b]	72.14

[a] Calculated as oleic acid from difference between iodine value and unsaturation accounted for by polyunsaturated fatty acids.
[b] By difference.

ditions used, GLC did not differentiate between *cis* and *trans* isomers or between conjugated and nonconjugated unsaturated acid esters. Therefore, the individual monoethenoid ester peaks probably include small amounts of unresolved *trans* isomers (13). Similarly, the GLC data for nonconjugated diene (C-18$^{2=}$ peak) and triene (C-18$^{3=}$ peak) probably include *trans* isomers of both nonconjugated and conjugated C-18 dienes, in addition to traces of C-19 and C-20 monoethenoid constituents (22, 24).

Table 5 shows that the GLC method provides a convenient way of comparing the polyunsaturated acid composition of different milk fats. The total of the C-18$^{2=}$ and C-18$^{3=}$ percentages closely approximates the total polyunsaturated fatty acids as determined by spectrophotometry. The GLC technique is quicker and less difficult, but the spectrophotometric method is sensitive to trace amounts of polyethenoid acids and differentiates between conjugated and nonconjugated isomers. James (14) recently described a more complicated GLC method, using two chemically distinct types of liquid phase to determine the degree of unsaturation and the chain length of long-chain fatty acids of unknown structure.

TABLE 6

Fatty acid composition of milk fat as determined by methyl ester fractionation and GLC

	Mole per cent of fatty acids					
	Ester fractionation		GLC			
	Jack and Henderson (12)	Hansen (6)	Hawke (9)	Samuels *et al.* (21)	Present study	
Component	Iodine value 32.4	Iodine value 39.6	Iodine value	Iodine value 35.0	Iodine value 35.8	Iodine value 27.4
C-2	....	....	0.3	....	....	....
C-4	9.2	10.5	9.1	2.9	6.7	8.7
C-6	2.8	3.9	3.9	3.6	3.5	4.8
C-8	2.7	1.8	1.8	2.4	1.7	2.2
C-10	3.5	3.3	1.4	4.1	2.9	4.3
C-10$^{1=}$	0.3	0.3	....	0.5[b]	0.4	0.7
C-12	5.2	3.8	2.9	4.4	2.5	3.4
C-12$^{1=}$	0.2	0.3	....	....	0.2	0.2
C-13	....	....	....	T.	0.2	0.2
C-14^{iso}	....	....	....	T.	0.1	0.1
C-14	14.8	9.8	9.6	12.6	8.3	13.2
C-14$^{1=}$	1.5	1.2	2.0	1.7	1.8	1.9
C-15	....	....	....	1.7	1.0	1.2
C-16^{iso}	....	....	....	0.5	0.2	0.1
C-16	27.2	23.3	23.3	29.3	27.7	19.7
C-16$^{1=}$	5.2	1.9	1.7	2.9	2.8	2.5
C-17^{iso}	....	....	....	....	0.9	0.8
C-17	....	....	....	0.7	0.8	0.7
C-18^{iso}	....	....	....	....	0.5	0.3
C-18	8.5	11.6[a]	13.9	9.4	10.1	12.3
C-18$^{1=}$	15.3	}	}	20.6	25.0	20.1
C-18$^{2=}$	1.7	} 26.8	} 26.6	1.5	1.9	2.0
C-18$^{3=}$	....	}	}	1.2	1.1	0.7
C-20	1.2	....	1.0	....	....	....
C-20 unsaturated	0.7	1.6	1.2	....	....	....

[a] C-18 plus C-20 saturated.

[b] Reported as C-11.

Fatty acid composition of milk fat as determined by methyl ester fractionation and GLC. Table 6 compares analyses by ester fractionation with data obtained by GLC. There is good agreement of the major constituents between the two methods, but GLC gives more detailed information on the minor components. The GLC procedure is also simpler and quicker than ester distillation.

Hawke's results do not include the proportions of odd-numbered and branched-chain acids, which were obtained in the present study, but do include C-20 saturated and unsaturated components. These were not resolved with the routine method employed in the present study. Even so, the method of Hawke is too complicated for routine analyses because steam-volatile acids as well as both normal and hydrogenated fatty acid esters must be prepared, and the different mixtures require two different liquid phases and methods of detection.

Samuels *et al.* (21) found a number of odd-numbered and branched-chain acids, but the reported mole per cent of butyric is obviously too low. Information in their paper is insufficient for comparison of their GLC method with the present one.

The two analyses from the present study are representative of a large number carried out in a study of the effect of feed on fatty acid composition of milk fat (26). Contrary to the respective results of Hawke and of Samuels *et al.*, no evidence was obtained for the presence of C-2 or C-11 acids.

CONCLUSIONS

A GLC method has been developed for the quantitative fatty acid analysis of milk fat. The procedure involves the preparation of methyl esters of the fatty acids by methanolysis and their GLC separation with diethylene glycol succinate as liquid phase. Correction factors are determined under the conditions used in the actual analysis. The accuracy of the method is superior to previous ester fractionation techniques and it yields additional data on the minor-component fatty acids. Although the method is useful for comparing the polyunsaturated fatty acid compositions of different milk fats, conventional spectrophotometric analyses give more detailed information on conjugated and nonconjugated components. Previous difficulties in the routine application of GLC to milk fat analysis have been largely overcome, and readily available commercial GLC apparatus is employed. The method described provides a more simple and precise way of analyzing the fatty acid composition of milk fat than is currently possible by any other means.

ACKNOWLEDGMENTS

This study was supported in part by funds from the California Dairy Industry Advisory Board. Miss T. Dairiki carried out most of the GLC analyses, and R. R. Lowry gave valuable technical assistance. The author is grateful to Dr. W. G. Jennings of this Department for valuable discussions.

AUTHOR'S NOTE

Since the manuscript was submitted for publication, two other methods for the GLC analysis of milk fat have been reported. S. Patton and associates (J. Dairy Sci., 43: 1187, 1350. 1960) have published a procedure that differs from the method described here both

in the preparation of methyl esters and in the GLC conditions employed to analyze them. They prepared a mixture of milk fat esters by saponification and esterification, separated the components by a capillary column, and detected them in an ionization chamber. Quantitative analyses by the two methods generally correspond, except that Patton's method did not determine methyl butyrate and the C-18$^{2=}$ and C-18$^{3=}$ peaks were not resolved. However, *cis* and *trans* C-18 monoenes were separated.

S. F. Herb and coworkers recently presented (34th fall meeting, American Oil Chemists' Society, October 16-19, 1960, New York, N. Y.) an analysis of milk fat by GLC with a thermal conductivity detector. They prepared methyl esters by direct methanolysis of the glycerides, then simplified the mixture by distillation and adsorption chromatography, and analyzed each of several fractions by GLC. These investigators identified over 60 fatty acids, including several minor ones with methyl ester retention volumes greater than methyl stearate.

The fatty acid composition of milk fat is extremely complex and the choice of a GLC method for its analysis is dependent on the nature of the research and the facilities available. Where the objective is to study the principal fatty acids of a number of samples, the method described here has certain advantages compared to Patton's method. For example, methyl ester preparation is simpler, the GLC equipment is less expensive, more rugged, and does not require complicated electronic circuitry. Butyric acid is included in the analysis and, if necessary, unknown peaks may be collected for further examination. However, as pointed out by Patton *et al.*, if only extremely small quantities of sample are available, the ionization detector and capillary columns are preferable. Where the minor component acids are of special interest, Herb's technique would be useful, provided the number of samples to be studied was few. Since ionization detectors permit the use of highly efficient capillary columns, improved columns may facilitate future routine analyses of the minor component acids of milk fat, especially those of C-18 and longer carbon chain length.

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AUTOXIDATION OF MILK LIPIDS. II. THE RELATIONSHIP OF SENSORY TO CHEMICAL METHODS FOR MEASURING THE OXIDIZED FLAVOR OF MILK FATS[1, 2]

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SUMMARY

Sixteen milk fat samples were collected from mixed herd milk over a 6-mo. period. Each sample was oxidized to a different stage yielding peroxide values ranging from 0 to 64 for the 16 samples. The TBA number, peroxide value, total saturated and unsaturated carbonyls, volatile saturated and unsaturated carbonyls, individual volatile monocarbonyls, and the absolute flavor threshold of each sample were determined. Statistical analyses of the data revealed that all chemical tests were highly correlated with each other. From 97 to 99% of the carbonyl-reactive material in oxidizing milk fats was nonvolatile. Relatively large amounts of nonvolatile carbonyls also were observed in fresh milk fat. The mean percentage of saturated carbonyls in the volatile monocarbonyls of 16 oxidized fats was 88.72.

The correlation coefficients between all of the chemical tests for oxidized flavor intensity and the reciprocal of the absolute flavor threshold (1/FTV) were significant at the 1% level, the volatile unsaturated carbonyls giving the highest, 0.996. Of the individual carbonyls, the correlation coefficient between non-2-enal and 1/FTV was the highest, 0.942.

Concentrations of individual volatile monocarbonyls, at the absolute flavor threshold of the fats, were in parts per billion. Based on reported data, the compounds occurred at marginal or subthreshold levels at the FTV of the fat. Multiple correlation coefficients support the view that the oxidized flavor, near the flavor threshold, is due to an additive effect of carbonyl compounds.

Most of the volatile monocarbonyls in oxidizing milk fats belong to four classes; namely, the n-alkanals, alk-2-enals, alk-2,4-dienals, and alkan-2-ones (6, 7, 8). The importance of each class or of individual compounds to oxidized flavor is not clear. Forss, Dunstone, and Stark (8) have attributed the fishy flavor of milk fat to n-hexanal, n-heptanal, hex-2-enal, and an unidentified metallic fraction. El-Negoumy and Hammond (7) reported that a single, unidentified compound is responsible for the oxidized flavor of butteroil. In reports from both of the above laboratories (7, 8), the qualitative composition of the volatile monocarbonyls is practically identical to that reported by Day and Lillard (6) for oxidized milk fats.

Day and Lillard (6) found that most of the volatile monocarbonyls were n-alkanals and alk-2-enals and that the qualitative composition of the mixture was the same regardless of the stage of fat oxidation. The reported flavor potency of these compounds (9, 12) and their predominance in the volatile fraction of oxidized fats led to the conclusions that they are important in

Received for publication September 8, 1960.

[1] Technical Paper No. 1349, Oregon Agricultural Experiment Station.

[2] This investigation was supported in part by PHS research grant No. RG-7017 from the Toxicology Section of the National Institutes of Health.

[3] Submitted in partial fulfillment of the requirement for the degree of Master of Science.

oxidized flavor and that the flavor is due to the combined effects of compounds (6).

The purpose of this investigation was to determine the quantitative composition of the volatile monocarbonyl mixtures of milk fat samples representing a wide range of oxidation and to ascertain the correlation of the various compounds with intensity of oxidized flavor. The correlation of chemical tests with flavor also was determined.

MATERIALS

Milk fats. Sixteen milk fat samples were collected from mixed herd milk over a 6-mo. period (January-June). These samples ranged in Iodine No. from 28 to 38. The samples were prepared as previously described (6) and were placed in quart milk bottles. To achieve a different level of oxidation for each of the 16 samples, it was necessary to expose samples for different lengths of time to diffuse laboratory light with the container seals loosened. Upon reaching a desired level of oxidation, the sample was stored at −20° C. until analyzed.

Solvents. Hexane was treated by passing it four times through a Celite-dinitrophenylhydrazine [4] column as described by Begemann and DeJong (3) and modified by Schwartz *et al.* (14). The treated hexane was then distilled and the 68 to 70° C. fraction was used. This treatment, while very effective, did not remove all of the carbonyl contaminants. The remaining contaminants were accounted for in control studies, as described previously (6).

[4] 2,4-Dinitrophenyl is hereinafter abbreviated DNP.

TABLE 1

Oxidation intensity of milk fats as measured by different tests

Sample No.	Carbonyls						TBA No.[b]	Peroxide value[c]	Flavor threshold[d]
	Total unsaturated[a]	Total saturated[a]	Total	Unsaturated volatiles[a]	Saturated volatiles[a]	Total volatiles[a]			
C[e]	0.61	12.1	12.71	0	0.05	0.05	0.24	0	40.0
1	0.99	8.76	9.75	0	0.11	0.11	0.51	0	5.70
2	0.91	7.47	8.38	0	0.24	0.24	0.53	0	3.10
3	2.94	14.05	16.99	0	0.28	0.28	3.92	11.98	0.80
4	2.83	15.90	18.73	0.03	0.26	0.29	4.82	12.54	0.42
5	4.53	10.00	14.53	0.02	0.20	0.22	3.92	16.69	0.41
6	3.55	14.77	18.32	0.03	0.26	0.29	5.90	20.91	0.39
7	6.24	12.03	18.27	0.04	0.24	0.28	6.57	18.66	0.35
8	3.73	16.08	19.81	0.05	0.35	0.40	6.17	13.35	0.34
9	4.87	13.95	18.82	0.02	0.42	0.44	7.86	17.30	0.30
10	4.26	10.26	14.52	0.04	0.17	0.21	5.53	13.33	0.25
11	5.92	9.43	15.35	0.05	0.42	0.47	9.67	17.80	0.25
12	13.25	28.25	41.50	0.12	0.48	0.60	30.90	64.60	0.17
13	5.04	15.01	20.05	0.04	0.41	0.45	15.03	26.45	0.16
14	3.68	16.45	20.13	0.06	0.43	0.49	17.05	28.65	0.15
15	7.91	17.30	25.21	0.07	0.55	0.62	18.02	29.72	0.14
16	6.79	23.15	29.94	0.13	0.82	0.95	27.03	45.72	0.12

[a] Expressed as millimoles of carbonyl per kilogram of fat.
[b] Expressed as milligrams of malonaldehyde per kilogram of fat.
[c] Expressed as milliequivalents of peroxide per kilogram of fat.
[d] Grams of oxidized fat per liter of 4% fat milk.
[e] Control sample of fresh fat.

Chloroform, ethanol, and benzene were treated by refluxing 3 hr. with DNP-hydrazine and trichloracetic acid, followed by distillation (4).

METHODS

Iodine absorption number. Hanus method (2).

Peroxide value. A.O.C.S. method (1).

2-Thiobarbituric Acid No. (TBA). The procedure was essentially that of Sinnhuber and Yu (15), except that the sample size ranged from 2 to 5 g. Values are expressed as milligrams of malonaldehyde per kilogram of fat.

Total carbonyl compounds. The total saturated and unsaturated carbonyls were determined on the intact fat by the procedure of Berry and McKerrigan (4). The values were expressed as millimoles of carbonyl per kilogram of fat.

Volatile carbonyl compounds. The volatile carbonyl compounds were isolated by means of the apparatus shown by Day and Lillard (6), except that a 250-ml. flask was used in place of Flask No. 3. One hundred–gram fat samples were warmed to 40° C. at 2-4 mm. Hg pressure and stripped with steam generated at the same pressures. The distillation was stopped after 50 ml. of water had passed from the steam generator. The distillate that collected on the cold fingers was allowed to melt and was added to 50 ml. of 5 *N* HCl saturated with DNP-hydrazine. The cold fingers were thoroughly rinsed with 50 ml. of ethanol and the rinse was added to the reaction mixture. After 24 hr., the DNP-hydrazones of the monocarbonyls were isolated by extracting five times with 25-ml. portions of hexane. The hexane extracts were combined, the solvent was removed at reduced pressure, and the dry DNP-hydrazones were then redissolved in 15 ml. of hexane. A 5-ml. aliquot was analyzed for millimoles of saturated and unsaturated carbonyls by the method of Berry and McKerrigan (4). Another 5-ml. aliquot was separated on a Method B, 15 g., 20-mm. by 25-cm. partition chromatography column (5) into the fractions shown in Figure 2 of a previous paper (6). The forerun fraction and Band 1 were combined, as were Bands 10 and 11, to give a total of ten fractions. The n-alkanals and alk-2-enals, previously shown to be the major constituents of each chromatographic fraction (6), were determined by the alcoholic KOH procedure (4). The values were converted to milligrams of carbonyl per kilogram of fat, using the molecular weights for the n-alkanal and alk-2-enal present in each fraction. Because the procedure does not differentiate n-alkanals from alkan-2-ones, the combined forerun and Band 1 were calculated as tridecanone and the traces of ketones in Bands 2, 3, 4, 5, 6, and 9 [see Figure 2, Reference (6)] were measured and expressed as n-alkanals.

Recovery studies. The efficiency of the distillation apparatus for removal of the volatile carbonyls from milk fat was ascertained by adding weighed amounts of authentic carbonyl compounds, at concentration levels encountered in oxidized fats, to 100 g. of fresh milk fat and determining their per cent recovery as described previously (6). The data presented in Tables 2 and 3 are corrected for recoveries on this basis.

Absolute flavor threshold determination. The procedure of Patton and

TABLE 2

Saturated volatile monocarbonyl compounds in autoxidized milk fats
Compounds (mg/kg fat)

Sample No.	C_{13}-one	C_{10}-al	C_9-al	C_8-al	C_7-al	C_6-al	C_5-al	C_4-al	C_3-al	C_2-al and C_1-al	Total
C[a]	0.88	0.10	0.04	0.14	1.29	0.24	0.71	0.04	2.83	1.90	8.17
1	1.38	0.14	0.17	0.28	0.32	0.70	2.23	0.39	2.07	8.69	16.37
2	1.38	0.53	0.21	0.65	0.44	1.36	0.42	1.37	1.89	14.84	23.09
3	1.30	0.60	1.25	0.93	2.99	4.58	5.59	0.67	3.00	16.98	37.89
4	2.58	1.15	1.07	1.58	15.39	3.91	4.41	0.57	2.73	13.84	47.23
5	2.82	1.12	1.05	1.29	12.92	3.73	2.72	0.51	1.01	14.29	41.46
6	2.37	1.18	1.29	1.51	13.91	3.75	3.72	0.59	1.27	10.84	40.43
7	2.50	1.97	1.80	1.92	17.53	4.81	4.27	0.57	1.01	10.57	46.95
8	3.79	0.95	1.63	1.91	17.17	4.42	6.36	0.38	3.65	20.84	61.10
9	1.87	0.82	1.83	1.87	21.07	5.53	9.44	0.67	3.39	16.01	62.50
10	3.50	0.58	1.62	1.86	13.46	4.53	6.30	0.49	1.89	14.87	49.10
11	1.66	1.28	0.55	1.97	9.66	5.14	8.07	0.85	1.47	21.23	51.88
12	3.36	3.06	5.39	6.98	22.24	24.42	7.82	1.35	5.05	19.42	99.09
13	2.09	0.94	2.58	3.01	12.61	9.86	9.53	1.06	3.86	19.18	64.72
14	1.23	1.05	2.78	3.87	13.46	10.55	9.65	1.35	3.34	28.57	75.95
15	2.95	1.74	3.24	4.19	17.30	10.67	15.94	1.82	5.04	36.28	99.17
16	1.60	2.35	5.08	3.96	11.91	10.84	10.94	1.15	4.45	15.02	67.30

[a] Control sample of fresh fat.

TABLE 3

Unsaturated volatile monocarbonyl compounds in autoxidized milk fats
Compounds (mg/kg fat)

Sample No.	C_{11}-enal	C_{10}-enal	C_9-enal	C_8-enal	C_7-enal	C_6-enal	C_5-enal	C_4-enal	Total
C[a]	0	0	0	0	0	0	0.005	0	0.005
1	0.02	0.02	0.04	0.06	0.06	0	0.01	0	0.21
2	0.03	0.07	0.04	0.05	0	0	0	0	0.19
3	0.22	0.06	1.02	0.18	0.38	0.16	0.38	0.17	2.57
4	0.51	0.61	1.11	0.14	0.91	0.27	0.69	0.31	4.55
5	0.38	0.48	1.13	0.44	0.82	0.02	0.32	0.17	3.76
6	0.35	0.37	1.24	0.44	0.75	0.19	0.28	0.07	3.72
7	0.80	0.85	1.69	0.87	1.74	0.49	0.60	0.12	7.16
8	0.74	0.90	2.15	0.46	1.16	0.02	0.96	0.21	6.60
9	0.50	0.25	3.24	0.27	1.52	0	1.19	0	6.97
10	1.51	0.98	2.35	0.47	1.32	0	0.58	0.31	7.52
11	0.69	1.00	4.53	0.29	1.85	0.10	1.12	0.37	9.95
12	1.41	2.68	7.26	2.33	2.81	1.17	3.21	0.78	21.65
13	0.96	0.69	5.61	0	1.99	0.01	1.44	0.51	11.21
14	0.93	1.88	7.16	0.21	2.59	0.67	1.87	0.57	15.88
15	1.32	1.66	7.88	0.67	2.92	0.32	2.40	0.33	17.50
16	2.08	2.82	6.24	0.65	1.47	1.26	3.80	1.23	19.55

[a] Control sample of fresh fat.

Josephson (13) was employed for measuring the absolute flavor threshold values (FTV) of the autoxidized milk fat samples. The sample was prepared as follows: The fat sample was melted at 55° C. and 12 g. was emulsified into 288 g. of fresh pasteurized skimmilk by means of a small hand-homogenizer. After adequate emulsification, the oxidized milk sample was diluted with fresh homogenized milk to the proper dilutions for the absolute flavor threshold determination. The range of dilutions varied with each oxidized sample, but always included concentrations well below to well above the flavor threshold. Fresh

homogenized milk was included in each evaluation as the control. The sample dilutions were expressed as grams of oxidized fat per liter of milk. It should be noted that in all cases, the milk samples, constituting a series of dilutions of oxidized fat, always contained 4% fat. For example, fat sample No. 4 (Table 1) was diluted in homogenized milk to levels of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 g. of oxidized fat per liter of milk. Each of the dilutions contained 40 g. of fat per liter of milk, but the amount of oxidized fat ranged from 0 to 1.0 g. per liter.

Judges were selected on their ability and consistency for detecting oxidized flavor. Five judges were used in the evaluation of a given fat sample. The percentage of positive responses vs. concentration of oxidized fat in the milk was plotted on graph paper. The 50% level of positive responses, expressed as grams of oxidized fat per liter of milk, was selected as the absolute flavor threshold value.

RESULTS AND DISCUSSION

Identification of hepta-2,4-dienal in oxidized milk fats. In previous work, Day and Lillard (6) were unable to find evidence for alk-2,4-dienals in oxidized milk fats. In their work, column partition chromatography was employed exclusively to fractionate the DNP-hydrazone mixture. Recent refinement of techniques (16), in which column partition chromatography is initially used to separate the DNP-hydrazones into multiple-class fractions, followed by separation of each fraction into its respective classes by the paper method of Gaddis and Ellis (10), has resulted in the identification of the DNP-hydrazone of hepta-2,4-dienal. This compound occurs at low concentration in column band No. 8, which is shown in Figure 2 of a previous paper (6). The hepta-2,4-dienal was obtained from the fraction as described and the following data established its identity: m.p. = 154-156° C.; mixture melting point = 153-156° C.; λ max. = 392 mμ; the chromatographic behavior of the unknown and the authentic DNP-hydrazone of hepta-2,4-dienal were the same.

Quantitative analysis. Table 1 shows data for fresh milk fat and 16 oxidized samples when analyzed by chemical and sensory methods. The samples are listed in the order of increasing oxidized flavor intensity as measured by the flavor threshold test. It will be noted that 99.6% of the carbonyl-reactive material of fresh fat and from 96 to 99% in oxidized fats were nonvolatile. Similar values were observed for the total malonaldehyde vs. the volatile malonaldehyde in the fats. Correlation coefficients between the various chemical tests revealed that all tests were highly correlated with each other. Of particular interest at this point were the correlation coefficients: peroxide value vs. TBA No. = 0.962; total carbonyl value vs. TBA = 0.961. The high correlation of these indirect tests with each other may be explained by the fact that reaction conditions for the tests are conducive to hydroperoxide decomposition and each test is largely measuring products of this reaction. This leads to the conclusion that the TBA and total carbonyl tests are largely indirect measures of lipid hydroperoxides and, like the peroxide determination, are measuring precursors or indicators of oxidized flavor. They are not a direct measure of the flavor compounds per se.

The quantities of individual carbonyl compounds that were found in the oxidized fat samples are given in Tables 2 and 3. In general, the concentration of volatile aldehydes increased congruently with oxidized flavor intensity. Changes in the methanal-ethanal fraction were erratic and may be attributable to the inability to consistently trap this fraction during distillation. Aside from the C_1 and C_2 fraction, n-heptanal occurred in highest concentration and was followed by n-hexanal and n-pentanal, respectively, in most fat samples, Table 2. Non-2-enal occurred in highest concentration of the unsaturated aldehydes shown in Table 3. The procedure of Berry and McKerrigan (4), when used on the DNP-hydrazones representing the complete mixture of the volatile carbonyls, did not reveal the presence of unsaturated carbonyls in fat samples 1, 2, and 3 of Table 1. However, the data in Table 3, where the mixture of DNP-hydrazones was separated prior to application of the alcoholic KOH procedure, show these compounds to be present.

A comparison of the mean percentage composition of the volatile carbonyl mixtures from the 16 oxidized fats, Table 4, with the data on fishy-flavored

TABLE 4

Mean percentage composition of the volatile monocarbonyl mixture from 16 oxidized milk fats

Saturated compounds	Mean	Standard deviation	Unsaturated compounds	Mean	Standard deviation
C_{12}-one	4.0	1.9	C_{11}-enal	1.1	0.8
C_{10}-al	1.8	0.8	C_{10}-enal	1.2	0.9
C_9-al	2.7	1.3	C_9-enal	4.2	2.5
C_8-al	3.3	3.0	C_8-enal	0.7	0.5
C_7-al	19.0	10.0	C_7-enal	1.9	0.9
C_6-al	9.2	4.0	C_6-enal	0.4	0.4
C_5-al	10.0	3.4	C_5-enal	1.5	1.1
C_4-al	1.4	0.7	C_7-dienal		
C_3-al	5.0	3.0	C_4-enal	0.4	0.4
$C_1 + C_2$-al	32.5	14.1			
Total	88.9		Total	11.4	

milk fat (8), indicates that variations in percentage composition of the flavor mixtures apparently have significant effects upon the sensory character of oxidized flavor(s). The major compounds found by Forss *et al.* (8) in their fishy-flavored isolate were n-heptanal 20%, n-hexanal 25%, non-2-enal 5%, hepta-2,4-dienal 8%, and hepta-2-one 10%, which differ from the data in Table 4 of this paper. The oxidized samples used in this investigation exhibited a typical oxidized flavor when examined in milk at concentrations near the absolute threshold of the flavor.

Correlation of chemical to sensory methods. The absolute flavor threshold values for the 16 oxidized fat samples are shown in Table 1. Analysis of the data of Table 1 revealed that an exponential relationship existed between the chemical tests for oxidized flavor intensity and the absolute flavor thresholds of the fats. The hyperbola, Curve A of Figure 1, depicts the relationship. A transformation of the flavor threshold values to their reciprocals (1/FTV) was employed for statistical treatment of the data. This relationship, for malonaldehyde vs. 1/FTV, is shown by Curve B of Figure 1.

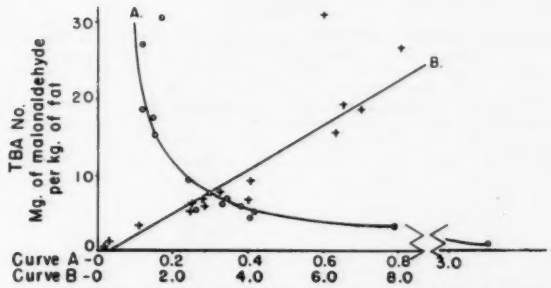

FIG. 1. Curve A. TBA No. vs. absolute FTV (grams of oxidized fat per liter of milk). Curve B. TBA No. vs. 1/FTV.

The correlation coefficients between the 1/FTV and all chemical tests employed were significant at the 1% level, Table 5. The surprisingly high correla-

TABLE 5

Correlation coefficients between reciprocal of flavor threshold and the various chemical tests for measuring the intensity of oxidized flavor

Chemical test	Correlation coefficient[a]
TBA No.	.887*
Peroxide value	.798*
Total unsaturated carbonyls	.666**
Total saturated carbonyls	.683**
Total carbonyls	.714**
Volatile unsaturated carbonyls	.996*
Volatile saturated carbonyls	.896*
Total volatile carbonyls	.887*
Sum of individual volatile unsaturated carbonyls	.948*

* Significant at 0.1% level.
** Significant at 1.0% level.
[a] Degrees of freedom = 15.

tion coefficients reported herein may be explained, in part, by the precision of the flavor threshold test and the heretofore-unreported relationship of flavor intensity to the chemical tests. The highest correlation coefficient was given by the volatile alk-2-enals. The correlation coefficient of .996 could have been influenced by the zero values obtained in the less oxidized samples, Table 1. However, the correlation coefficient of the total alk-2-enals for each fat, as taken from Table 3, with 1/FTV was 0.948. Holm, Wode, and Thome (11) also found a high correlation between these compounds and the oily flavor of butter. The high correlation of the total volatile carbonyls with oxidized flavor is in agreement with the findings of Berry and McKerrigan (4) for other fats and oils.

Correlation of individual carbonyls to oxidized flavor. The correlation coefficients for each volatile monocarbonyl compound of the 16 fats vs. 1/FTV are given in Table 6. Non-2-enal gave the highest correlation coefficient, 0.942. In general, the individual alk-2-enals gave higher correlations than the saturated aldehydes. This is in agreement with the aforementioned high correla-

TABLE 6

Correlation coefficients between reciprocal of flavor threshold and the individual volatile monocarbonyl compounds in oxidized milk fats

Compound	Correlation coefficient[a]	Compound	Correlation coefficient[a]
C_{11}-enal	.439	C_{13}-one	.151
C_{10}-enal	.858*	C_{10}-al	.604***
C_9-enal	.942*	C_9-al	.844*
C_8-enal	.372	C_8-al	.824*
C_7-enal	.866*	C_7-al	.426
C_6-enal	.660**	C_6-al	.725**
C_5-enal	.894*	C_5-al	.857*
C_4-enal	.619***	C_4-al	.294
		C_3-al	.505***
		$C_2 + C_1$-al	.455

* Significant at 0.1% level.
** Significant at 1% level.
*** Significant at 5% level.
[a] Degrees of freedom = 15.

tions of the total volatile unsaturated aldehydes with 1/FTV. Recently, Forss, Dunstone, and Stark (8) have reported that the significant compounds in fishy-flavored fat were n-hexanal, n-heptanal, hex-2-enal, and a metallic flavoring compound. In this investigation, the correlations of n-hexanal and hex-2-enal with flavor were significant at the 1% level. Heptanal, with the highest mean percentage concentration of the longer-chain carbonyls, Table 4, gave a correlation with 1/FTV of 0.426. In view of the high concentration of heptanal in the oxidized fats and its apparent flavor potency (12), it does not appear sound to discount it as an important component of the flavor, even though its statistical relationship to flavor intensity was insignificant.

Multiple correlation coefficients. To determine possible multiple effects of compounds on their correlation with 1/FTV, multiple correlation coefficients were calculated. The correlation coefficients of the C_4, C_8, and C_{11} alk-2-enals were 0.619, 0.372, and 0.439, respectively, whereas the multiple correlation coefficient of the combined values of these compounds with 1/FTV was 0.945. Other examples of multiple correlations where improvement in coefficients was observed were: heptanal + oct-2-enal, 0.512; ethanal + butanal + heptanal, 0.639. In each of the above cases, the multiple correlation was higher than the correlations for individual compounds as shown in Table 6. These data suggest that the typical oxidized flavor of milk fat is due to the multiple effects of compounds rather than to a single compound.

Concentration of carbonyl compounds at the absolute flavor threshold of oxidized flavor. The concentration of each carbonyl at the absolute flavor threshold of the fat sample was calculated by the formula: $\frac{\text{FTV}}{1{,}000} \cdot X = \text{milligrams}$ of carbonyl per liter of milk, where X = concentration of carbonyl per kilogram of fat as listed in Tables 2 and 3. The mean concentration of each carbonyl in the 16 fat samples at the absolute threshold of oxidized flavor, and the standard deviations, are shown in Table 7. All compounds occurred at parts per billion levels at the FTV of the fat. The limited data for flavor thresholds of

TABLE 7

Mean concentration of carbonyl compounds in oxidized milk fats at the absolute flavor threshold

Compound	Mean[a]	Standard deviation	Compound	Mean[a]	Standard deviation
C_{13}-one	1.4	1.9	C_{11}-enal	0.2	0.1
C_{10}-al	0.5	0.1	C_{10}-enal	0.2	0.1
C_9-al	0.6	0.2	C_9-enal	0.7	0.3
C_8-al	0.8	0.4	C_8-enal	0.1	0.1
C_7-al	3.6	1.9	C_7-enal	0.4	0.1
C_6-al	2.1	1.1	C_6-enal	0.1	0.1
C_5-al	2.6	2.8	C_5-enal	0.3	0.1
C_4-al	0.6	1.1	C_4-enal	0.1	0.1
C_3-al	1.8	0.8	Total	2.1	
$C_1 + C_2$-al	10.4	14.8			
Total	24.4				

[a] Expressed as parts per billion.

the various carbonyl compounds preclude definite conclusions relative to their organoleptic properties at the concentrations shown in Table 7. Based on reported threshold values for some of the compounds (9, 12), it would appear that in most cases the individual carbonyl compounds occur at marginal or subthreshold levels in oxidized milk fat, at the point where the flavor is perceptible 50% of the time (FTV). The data further indicate that at the absolute flavor threshold of the oxidized fats, the flavor may be due to the additive effects of compounds, each compound occurring at marginal or subthreshold levels, but the combined concentration equaling or exceeding the FTV of any one compound in the mixture. Compounds such as the C_7, C_8, and C_9 alk-2-enals, which are very similar in sensory character, may act in such a way. The high correlation coefficients of individual compounds with 1/FTV and the highly significant multiple correlations support this point.

It is recognized that most of the fat samples used in this investigation were oxidized beyond the intensity normally encountered in dairy products. This was done purposely, in order to ascertain the influence of a wide range of oxidative stages on the relation of chemical to sensory measurement of oxidized flavor. While the experiment was not designed to distinguish differences in the sensory character of oxidized fats, no pronounced differences were noted when the fats were evaluated in milk at the concentrations necessary for flavor threshold determinations.

ACKNOWLEDGMENTS

The authors thank T. C. Yu for his assistance in the identification of hepta-2,4-dienal and L. D. Calvin for his advice during the statistical analyses of the data.

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HIGH-TEMPERATURE SHORT-TIME STERILIZED EVAPORATED MILK. III. THE INFLUENCE OF THE LIPID PHASE ON HEAT AND STORAGE STABILITY

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SUMMARY

Laboratory experiments in which high temperature–short time sterilization of concentrated milk was carried out under static conditions showed that heat and storage stability were at times adversely affected by the presence of a lipid phase. Three types of milk were encountered. The predominant type (if forewarming was employed) consisted of milks in which the fat phase behaved as an inert phase, that is, the processed whole and the corresponding processed skimmilks were heat-stable and resisted gelation during storage to approximately the same degree. On occasions, a type of milk was encountered in which the processed whole but not the corresponding processed skimmilk coagulated during sterilization. On other occasions, a type of milk was met in which the processed whole milk had a slightly greater shelf-life than the corresponding processed skimmilk. Exchanging the fat globules belonging to a heat-stable for those in a heat-labile milk tended to reverse the order of stability. Substitution of fat for an equal weight of protein showed that both heat stability and storage stability were significantly decreased by the substitution. Milks prone to coagulate because of an unstable lipid phase were stabilized to some extent by forewarming against both heat coagulation and gelation during storage. Mixing of forewarmed and unforewarmed milks resulted in composite milks with heat and storage stabilities intermediate between those of the unmixed milks. Milks reconstituted from butter oil and skimmilk were stabilized by the addition of fat-soluble interfacially active mixtures to the butter oil.

Sterilization under static conditions yields results which, supplementing the results of sterilization under turbulent canditions, allows one to isolate the effect of heat from the combined effect of heat and turbulent flow.

The effect of variations in milk composition and fat concentration on body changes in evaporated milk has been discussed by a number of investigators. Bell, Curran, and Evans (1) state: "During high-short sterilization, there is little opportunity to control body, and through a heavier body, the extent of fat separation." Tarassuk and Tamsma (9), comparing the rate of gelation of concentrated whole and skimmilks, conclude: "The accelerated rate of gelation with increased concentration is entirely associated with increased concentration of the solids-not-fat fraction."

The results of laboratory investigations on milk held in situ in thin-walled capillary tubes during sterilization have been found at times to be at variance with the views just cited. The fat globules in concentrated whole milk appear often to possess the capacity of aggregating not only among themselves but also with the suspended proteins, thereby accelerating gel formation during storage. The data pertaining to these observations form the basis of the present report. An attempt will be made to reconcile divergent views.

Received for publication July 22, 1960.

EXPERIMENTAL PROCEDURE

The micro-techniques described previously were employed throughout this study (6). Samples were forewarmed at the temperature of boiling water for a total elapsed time (bringing up and holding) of 17 min. The forewarmed milk loaded in microviscometers was sterilized at 137.4° for 15 sec. The viscometers were of the Höppler falling-ball type.[1] Storage life was arbitrarily taken as the number of days elapsing before the viscosity of the sample acquired a viscosity value equal to twice the minimum value observed during storage.

In one experiment, samples were emulsified and homogenized with an improvised Diesel Engine Nozzle Tester and nozzle combination, the performance characteristics of which are discussed in another paper (7).

Unless otherwise stated, samples were rotated slowly during storage at 30°, thereby minimizing creaming and sedimentation.

Homogenization efficiency was determined turbidimetrically by the method of Deackoff and Rees (4). Emulsification efficiency was also determined turbidimetrically. However, the turbidity index was taken not as the transmittance of a diluted sample at 1,020 mμ but rather as the ratio between the transmittances at 1,020 and 500 mμ.

RESULTS

Heat stability of high-temperature–short time (HTST) evaporated milk. The application of the laboratory techniques described in preceding papers to the study of HT-ST evaporated milk led immediately to an unusual result. Fresh pasteurized market milk (3.5% fat and 12.5% total solids) which had been homogenized at 2,000 lb. p.s.i. and rehomogenized at 300 lb. p.s.i., was forewarmed and concentrated to 26% solids, and the concentrate was finally sterilized at 137.4° for 15 sec. The milk coagulated during sterilization. Neither lowering of the holding time from 15 to 2 sec. nor adding disodium hydrogen phosphate brought about improvements in stability. In taste, appearance, and in pH and acidity the milk was typical of fresh, wholesome market milk.

A second sample of unstable homogenized milk (B) from the same source as that used above and purchased in the same month (April) was compared with a stable milk (A) obtained from an independent source. Portions of each milk were centrifuged for 10 min. at approximately 25,000 r.p.m. in a Servall centrifuge. Separation was incomplete, the resulting partially separated skimmilks from B and A containing, respectively, 1.0 and 0.62% fat. In addition free fat formed in each of the creams. The cream layers were separated from their underlying skimmilks (including whatever protein may have been sedimented) and the creams and the contents of the underlying layers belonging to each sample were pooled. Fat concentrations were determined and three milks were prepared by combining (1) cream from A with skimmilk from B; (2) cream

[1] The mention of trade names in this paper is for identification and implies no endorsement of the products.

from A with skimmilk from A; (3) cream from B with skimmilk from B. The reconstituted milks were blended at 50° in a Waring Blendor, after which the blended milks were forewarmed and concentrated to 26% solids. Viscosity characteristics of the concentrate before and after sterilization are given in Table 1. Exchanging the major portion of the fat phase of the milk labile to

TABLE 1

Stabilization brought about by transfer of cream from heat-stable milk to skim from heat-labile milk

Sample	Viscosity before sterilization (centipoises)	Viscosity after sterilization for intervals of: 10 sec.	15 sec.	20 sec.	40 sec.
Cream (A) plus Skim (A)	3.5	4.5	....	8.7	....
Cream (B) plus Skim (B)	3.6	....[a]			
Cream (A) plus Skim (B)	3.6	8.0	9.1	10.4	21.6

Milks sterilized at 137.4° C. for 15 sec.
(A) Refers to source—USDA herd—Beltsville, Maryland.
(B) Refers to source—local dairy.
[a] Coagulation occurs during sterilization.

heat for an equivalent quantity of the fat phase belonging to the heat-stable milk increased the heat stability of the labile milk to such a degree that the viscosity values of the heat-stable and the stabilized heat-labile milks were of the same order of magnitude over a wide range of sterilizing times.

Skimmilks corresponding to the whole milks discussed above were obtained by centrifugal separation of the unhomogenized whole milks, and concentrated. The concentrates were remarkably heat-stable. Even after treatment for 11 min. at sterilizing temperature (137.4°) the concentrated skimmilks, one corresponding to the heat-labile the other to the heat-stable concentrated whole milk, showed not the slightest suggestion of graininess, instability, or heavy body. Data illustrating the viscosity characteristics acquired by the two skimmilk concentrates as a result of heat treatment at 137.4° are given in Table 2.

The data show that the differences in heat lability are associated with differences, presumably in composition, between the lipid phases. To test this

TABLE 2

Comparison of the heat stability of two concentrated skimmilks. The corresponding whole milk concentrates differed widely in stability (See Table 1)

Sterilization time at 137.4° C. (sec.)	Viscosities after sterilization (centipoises) Skimmilk[a]	Skimmilk[b]
10	3.8	4.1
60	3.4	4.1
120	3.2	4.1
300	3.3	5.2
660	4.0	8.3

[a] Skimmilk concentrated to 19% solids; skim from Beltsville heat-stable whole milk.
[b] Skimmilk concentrated to 19% solids; skim from purchased heat-labile whole milk.

assumption the following experiment was performed: To each of 50-g. samples of filtered melted butter oil, liquid food emulsifier was added to yield solutions containing 0, 2, 4, and 6% emulsifier. The emulsifier was a commercial product with a wide use in the ice cream industry. According to the manufacturer, it consisted of a mixture of mono- and diglycerides of fat-forming fatty acids, the monoglycerides of which comprised 55% of the total glycerides. The product dissolved freely in butter oil to yield clear solution. Portions of each of the butter oil solutions were injected at a temperature of 70° and an injection pressure of 4,800 lb. p.s.i. into 100-ml. samples of skimmilk, to yield four milks containing slightly more than 4% fat and varying only in emulsifier concentration. Following fat analysis by the Babcock method, in which the emulsifier separates with the fat phase, the milks were adjusted to contain 4% fat. The 4% milks were pasteurized by heating to 70° C., and the pasteurized milks were cooled to 20° C. and stored in a refrigerator at approximately 5° C. Cream-line measurements were made after two days. Seventy-five-milliliter samples were homogenized at 3,200 lb. p.s.i. and 70° and rehomogenized, and the homogenized milks were concentrated to contain 27% solids. Correspondingly, a sample of skimmilk was concentrated to contain 19.6% solids. Homogenization indices were determined. Samples were loaded into viscometers and sterilized. Tables 3 and 4 contain data bearing on this experiment. More

TABLE 3

Some properties of milk reconstituted from butter oil, stabilizer, and skimmilk. These milks were subsequently sterilized and viscosity changes were followed (See Table 4)

Fat	Stabilizer	Cream line[a]	Emulsification index[b]	Homogenization index[c]
(%)	*(g/100 g. fat)*	*(%)*		*(% transmittance)*
4.0	0	24	1.46	67
4.0	2	20	1.52	70
4.0	4	11	1.59	72.5
4.0	6	9	1.66	75.5

[a] Per cent after three days of storage at 5° of total milk column height.
[b] Ratio between transmittances at 1,020 and 500 mμ of milk diluted with 0.1 *N* NH_3 to contain 0.0137% fat. Indices obtained after injection of fat into skimmilk.
[c] Transmittance of diluted milk at 1,020 mμ. Indices obtained after homogenization of milk, according to method of Deackoff and Rees (4).

efficient emulsification on injection of butter oil into skimmilk was associated with the use of fat-soluble emulsifier. Effectiveness of emulsification increased as the emulsifier concentration was increased from 2 to 6 g/100 g. fat.

Similarly increased homogenization efficiency attended the use of emulsifier. The incorporation of 6 g. emulsifier per 100 g. fat yielded a milk which, when homogenized, had a homogenization index of 75.5%. In terms of conventional homogenization practice, it would require approximately an additional 1,000 lb. p.s.i. pressure to bring the homogenization index of the milk lacking emulsifier to the 75.5% level.

Milk containing no emulsifier suffered pronounced coagulation on sterili-

TABLE 4

Influence of fat soluble stabilizer on storage life and viscosity characteristics of milks reconstituted from skimmilk, butteroil, and varying amounts of stabilizer. (See Table 3 for composition data)

Emulsifier concn. (g. per 100 g. fat)	Viscosity before sterilization (centipoises)	Viscosity after sterilization		Elapsed time before viscosity rises (days)	Storage life (days)
		Immediately (centipoises)	Minimum (centipoises)		
0	5.2	Coagulation	....	....	0
2	5.6	158	45	33	54
4	5.3	10.5	6.8	34	70
6	5.1	9.7	6.4	32	71
0[a]	3.8	4.3	3.3	41	78

Milks sterilized at 137.4° C. for 15 sec.
[a] Skimmilk.

zation. The coagula dispersed on stirring to yield a plastic suspension which did not thin out on storage. The concentrate containing 2 g. emulsifier per 100 g. fat thickened as a result of sterilization. The sterile heavy product exhibited considerable false body.[2] Increasing the emulsifier concentration to 4 g. per 100 g. fat relieved the tendency to coagulate. A thin-bodied sterile product was obtained, the viscosity characteristics and the storage life of which approached that of the sterile concentrated skimmilk control. Increasing emulsifier concentration further brought with it only slight changes. Not only heat stability but also storage life increased significantly with increasing modification of the composition of the fat phase which, when it contained 4-6% emulsifier, behaved very nearly as an inert phase.

The possibility suggested itself that on occasion milks might be found in which the stability of the fat phase would be reflected in the greater storage stability of the concentrated whole, compared with the corresponding concentrated skimmilk. The results described below are in accord with this view.

Exchange of phases belonging to two stable milks. The following experiment was originally designed to demonstrate further the changes brought about by exchanging the phases of labile and stable unhomogenized milks. Unfortunately, the milk which judging from the source of supply and the date of purchase might have been expected to be unstable proved quite stable. Nevertheless, the effect of exchanging phases is quite interesting. Four milks were prepared from raw whole milks A and B, each from an independent source. The milks contained cream and skimmilk in the following combinations: (1) Cream A and Skimmilk A; (2) Cream B and Skimmilk B; (3) Cream A and Skimmilk B; and (4) Cream B and Skimmilk A. The milks standardized to contain 3.9% fat were pasteurized at 63° for 15 min. and homogenized in two stages at 2,000 and 500 p.s.i.g., respectively. The homogenized milks were forewarmed at 100° for 17 min. and concentrated to 26% solids. Skimmilks were forewarmed and

[2] The body of evaporated milk immediately after sterilization is usually heavier than the body after short storage. Body (as measured by viscosity) which is lost in storage is referred to in the evaporated milk industry as false body.

concentrated to 18.2% solids. All milks were sterilized at 137.4° for 15 sec. and stored at room temperature.

Examination of the data in Table 5 shows that the evaporated milks con-

TABLE 5
Slight prolongation of storage life brought about by a stable fat phase

Cream source	Skimmilk source	Viscosity before sterilization (centipoises)	Viscosity after sterilization (centipoises)		Storage life (days)
			Immediate	Minimum	
A	A	3.6	4.8	4.3	130
B	B	3.5	5.3	5.0	106
A	B	3.6	5.1	4.1	123
B	A	3.5	5.1	4.9	106
....	A	3.0	2.8	2.6	106
....	B	3.1	3.5	3.4	112

Milks sterilized at 137.4° C. for 15 sec.
A. Milk from USDA herd, Beltsville, Maryland.
B. Milk from local dairy.

taining Cream A had a significantly longer storage life than milks containing Cream B, regardless of the source from which the skimmilk was derived. The milks containing Cream A also had a significantly longer life, although not in a practical sense, than either of the evaporated skimmilks. All milks were rather thin-bodied. The viscosity at any moment following sterilization appeared to reflect opposing tendencies, one tending to increase, the other to decrease viscosity.

Effect of substituting fat for proteins. In the experiment described below, milk fat was substituted for an equal weight of milk protein. To effectuate this exchange a synthetic milk serum was employed with the following composition:

Substance	grams per liter
Lactose	50
Citric Acid H_2O	0.46
KCl	2.8
$Na_2HPO_4 \cdot {}_2H_2O$	2.24

The pH was adjusted to pH 6.6, the pH of the milk. The resulting solution had an ionic strength of 0.075, compared with a cited value (10) of 0.073 for milk ultra-filtrate.

The following milks were prepared:

1. Milk containing 3.5% fat and 3.4% protein.
2. Milk containing 4.0% fat and 2.9% protein.
3. Milk containing 4.5% fat and 2.4% protein.

The milks were flash-heated to 71° C. Portions of each were homogenized in two stages, at 2,500 then at 500 lb/sq in. Portions were also homogenized at 7,500-500 lb/sq in. One part of each of the homogenized milks was forewarmed, the remaining part was not. All milks were concentrated to 26% solids and sterilized at 137.4° for 15 sec. The data in Tables 6 and 7 show that

TABLE 6

Influence of substituting fat for protein on viscosity characteristics and storage life of high temperature-short time sterile evaporated milks prepared from unforewarmed milks

Viscosity before sterilization (centipoises)	Viscosity after sterilization		Time for viscosity to reach minimum (days)	Storage life (days)
	Immediately (centipoises)	Minimum (centipoises)		
3.8[a]	5.2	5.0	10	120 ± 10
3.5[b]	4.7	4.5	2	75 ± 4
3.2[c]	4.1	4.1	0	65 ± 2

Milks sterilized at 137.4° C. for 15 sec.
[a] Sample contained 7.3% fat and 7.1% protein.
[b] Sample contained 8.3% fat and 6.0% protein.
[c] Sample contained 9.3% fat and 5.0% protein.
Samples run in quadruplicate. Milks homogenized in two stages at 2,500 and 500 p.s.i.g. Homogenization temp., 71°. Milks homogenized at 7,500 p.s.i.g. coagulated during sterilization.

TABLE 7

Influence of substituting fat for protein on viscosity characteristics and storage life of high temperature-short time sterile evaporated milks prepared from forewarmed milks

Viscosity before sterilization (centipoises)	Viscosity after sterilization		Time for viscosity to reach minimum (days)	Storage life (days)
	Immediately (centipoises)	Minimum (centipoises)		
4.0[a]	6.3	5.2	0–10	133 ± 10
3.4[b]	4.1	4.0	0– 1	130 ± 10
3.8[c]	5.6	5.2	0–10	108 ± 3
3.4[d]	4.2	4.1	0– 1	119 ± 1
3.2[e]	4.8	4.4	0–10	88 ± 0
3.1[f]	4.6	4.6	0– 1	79 ± 0

Milks sterilized at 137.4° C. for 15 sec.
[a, b] Samples contained 7.3% fat and 7.1% protein.
[c, d] Samples contained 8.3% fat and 6.0% protein.
[e, f] Samples contained 9.3% fat and 5.0% protein.
Samples run in duplicate. Milks for [a, c] and [e] were homogenized in two stages at 2,500 and 500 p.s.i.g. Milks for [b, d] and [f] were homogenized in two stages at 7,500 and 500 p.s.i.g.

the substitution of fat for an equal weight of protein brought about a significant decrease in storage life. Concentrates derived from unforewarmed, high-pressure (7,500-500 lb/sq in.) homogenized milks were heat-labile and showed coagulation damage immediately following sterilization. Damage was greatest in the high-fat, low-protein milk. The storage life of concentrates derived from the low-pressure (2,500-500 p.s.i.g.) homogenized milks increased from 65 to 120 days as the ratio between protein and fat increased from 0.54 to 0.97.

Forewarming effected a general improvement in storage quality. However, the relative order of storage life with respect to changes in the protein to fat ratio remained unchanged. Forewarming served to erase the storage life differences between the low- and high-pressure homogenized milks. The high degree of heat-lability in concentrates derived from the unforewarmed, high-pressure homogenized milks failed to manifest itself in concentrates derived from forewarmed milks.

Mixing of heat-labile and stable milks. Forewarming stabilizes milk both against heat coagulation and gelation during storage. The increase in stability is the resultant of the following changes: (1) An increase in the stability of the lipid phase; (2) an increase in the stability of the protein phase; and (3) a composite increase arising out of interaction. Mixing of a forewarmed and unforewarmed milk results in a composite milk with heat and storage stabilities intermediate between those of the unmixed milks. Inasmuch as in the experiments described below the lability of the unforewarmed milk was associated largely with the lability of its lipid phase, the change in stability on mixing is likewise associated largely with changes in the proportion of the heat-labile lipid phase.

Milk containing 3.5% fat was homogenized in two stages at 7,500 and 500 lb. per sq. in. A portion was forewarmed, and samples of forewarmed and unforewarmed milks were concentrated to contain 26% solids. The concentrates were combined in varying proportions and sterilized at 137.4° for 15 sec. Viscosity was determined immediately after sterilization and during storage. The data are plotted in Figure 1. Storage life increased in direct proportion to the concentration of forewarmed milk. As the proportion of forewarmed milk was increased, the viscosity decreased, quite rapidly with each additional

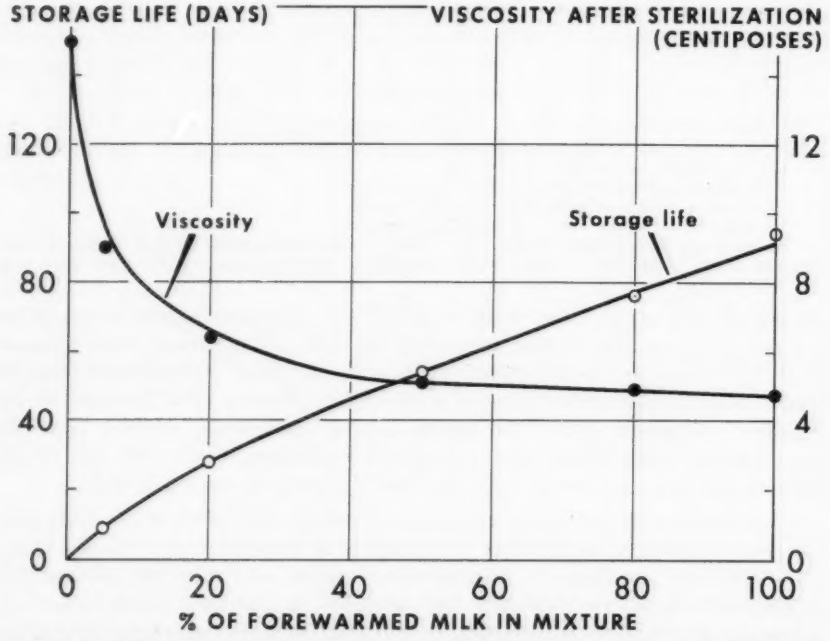

FIG. 1. Effect of mixing concentrates from forewarmed and unforewarmed milks on storage life and viscosity of sterile products. Milk homogenized in two stages at 7,500 and 500 p.s.i.g.

increment at relatively low concentrations of forewarmed milk and more slowly at high concentrations. The viscosity of samples containing small concentrations of forewarmed milk increased during the first few days of storage in inverse proportion to the concentration of forewarmed milk. No lag phase (period of no increase in viscosity) was observed.

DISCUSSION

Perhaps a suitable starting point for discussion is to reconcile the observation made in pilot plant and plant practice that only thin-bodied products are produced in HT-ST methods, with the results disclosed in this paper. The difference is very likely a result of the static sterilization and cooling conditions employed in the laboratory, compared with the conditions of turbulent flow employed in pilot plant practice. Thin body is a consequence not only of a much milder heat treatment in high temperature–short time, compared with long hold methods, but also a consequence of the extremely high degree of agitation which milk experiences in turbulent flow. Heavy body occasioned by clusters of fat globules and/or clusters comprising fat globules and protein micelles are easily reduced at the rate of shear attending either the passage of milk at high velocities through small tubes or the exposure of milk to steam jets.

With respect to the differences which prevail between plant and laboratory techniques, the employment of static sterilization and cooling methods may well yield results which, supplementing the results obtained in the plant, allow one to isolate the effect of heat from the combined effects of heat and turbulent flow. This correlation is especially important, inasmuch as the laboratory data indicate that storage life is related in some manner with heat lability brought about by an unstable lipid phase.

Thin-bodied sterile skimmilk concentrates are produced as a rule, probably without exception, in high temperature–short time processing as carried out in the plant. Heavy-bodied and coagulated products, however, are obtained in the laboratory processing of whole milk. Although their occurrence is the exception rather than the rule, these products nevertheless are obtained quite frequently.

On theoretical grounds, a wholly inert fat phase in milk is difficult to conceive. With respect to coagulation kinetics, the properties of the interfacial region of the fat globules would be a weighty factor. The fat-globules in unhomogenized milk are surrounded by a membrane (5, 8). Homogenization destroys the native membrane, giving rise to an interfacial region possessing a different character. Brunner *et al.* (2, 3), examining the differences in protein composition between the interfacial regions of unhomogenized and homogenized milks, concluded that in contradistinction to the differences in unhomogenized milk between plasma and interface proteins, there was a close resemblance between the corresponding proteins in homogenized milk.

The electrokinetic potentials of fat globules from homogenized and unhomogenized milks are substantially the same. The isoelectric points, likewise, differ very little and correspond quite closely with that of casein.

King (5) has constructed a picture of the interfacial region in which nonproteins, predominantly phospholipids, occur in juxtaposition with proteins. This construction justifies the assumption that a fat globule would behave under certain specified conditions as a protein particle, modified by the presence of phospholipids and cholesterol in the interfacial region.

Viewed dynamically, the fat globule at times would behave as a large protein particle activated as a result of orientation, and at other times as an activated nonprotein particle. The apparent inertness[3] of the fat globules in HT-ST processing is a surprising result, for they comprise about one-half of the total coagulable material in milk. If their interfacial composition prompts the fat globules to behave as protein, coagulation should be accelerated greatly both during sterilization and storage. That the fat phase behaves as a quasi-inert phase at all must mean that it is acting in virtue of the nonproteins (phospholipids and cholesterol) present in the interfacial region, thereby acquiring the virtues of a truly inert phase. Such a phase would actually limit the development of graininess by hindering free diffusion, in much the same way as the fat phase retards the development of large ice crystals in ice cream manufacture. It is in virtue of these two antagonistic properties—the ability to function either as protein or as a truly inert emulsoid—that the fat globules influence coagulation phenomena in high temperature–short time sterile evaporated milk. This view is strongly supported not only by the experiments of this paper but by experiments which will be reported in the next paper of the series.

The experimental data pertaining to the effect on viscosity and storage life of mixing forewarmed and unforewarmed milks show that a lag or induction period is not necessarily a prerequisite for the development of a gel structure. The curd formed during the storage of HT-ST sterile milk concentrates resembles in appearance a rennet-produced curd. There is a strong temptation to conclude by analogy that protein modification through the splitting off of low molecular weight peptides probably precedes coagulation and gelling. The rate-determining factor would be the rate at which the proteins are cleaved. The occurrence of an induction period, together with the observation that storage life is independent of added calcium, would support this view. However, it would require inordinate amounts of enzyme in unforewarmed sterile concentrates to produce coagulation in the short times observed experimentally.

No special assumptions based on protein modification are required to account for the relatively short storage life of some HT-ST milks. If analogies are needed, perhaps the most cogent one is the analogy between coagulation in superheated condensed milk and in HT-ST evaporated milk. No storage period is involved in the preparation of heavy-bodied superheated milk.

[3] True inertness refers to the nonparticipation in coagulation kinetics of the fat phase. The results in this paper show that the fat phase does participate in coagulation kinetics. Two opposing tendencies presumably operate simultaneously—one favoring the coagulation process, the other hindering it. The expression "quasi-inertness" is used in this paper to indicate a seeming inertness caused by the predominance of the tendency which hinders the coagulation process.

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TRISATURATED GLYCERIDES OF MILK FAT[1]

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SUMMARY

A number of samples of milk fat have been analyzed for trisaturated glyceride by the mercaptoacetic acid method. The amount of trisaturated glyceride varied from 21.5 to 32.0% by weight. The amount of trisaturated glyceride was found to agree well with the amount calculated by random distribution. The fatty acid composition of the trisaturated glyceride was determined by gas phase chromatography and compared with that of the whole fat. The results show that there is no preferential selection or exclusion of any of the major saturated fatty acids from the trisaturated glycerides. These and other recently determined structural features of milk fat can best be explained in terms of the limited random distribution theory of Vander Wal. It was also found that the relation among the melting point of a fat and the melting point and amount of trisaturated glyceride, which has been found to hold for many fats, does not hold for milk fat. It is shown that this means that the heat of fusion of the last glyceride in the fat to melt changes from sample to sample.

A number of earlier studies on the trisaturated glycerides of milk fat have been reviewed by Hilditch (11). These were based on the oxidation of milk fat with potassium permanganate and the separation of the trisaturated glycerides from the azelaoglycerides by extraction of the soaps. It was found that there was a correlation between the amount of trisaturated glyceride in milk fat and the amount calculated by random distribution. Moreover, when the fatty acids present in the trisaturated glycerides were analyzed by fractional distillation of the methyl esters, it was found that the fatty acids were present in amounts roughly proportional to their concentration in the whole fat. Although these facts are most readily explained by assuming a random distribution of the fatty acids in milk fat, Hilditch has proposed alternative schemes to account for the distribution (10, 11).

The permanganate oxidation technique is subject to considerable error. Ting (21) was unable to get reproducible results using it on milk fat. Kartha (15) has claimed that alkaline products are produced which cause hydrolysis of the azelaoglycerides. Eshelman and Hammond (7) have confirmed that permanganate oxidation of fats by Hilditch's technique (12) causes considerable hydrolysis. They have also found that Kartha's technique leads to side reactions. Aside from any hydrolysis that may occur, alkaline products such as potassium hydroxide and potassium carbonate that might accumulate during the oxidation have been shown to be excellent interesterification catalysts (9). Thus, one can not be sure if the apparent tendency toward random composition of milk fat

Received for publication May 14, 1960.

[1] Journal Paper Number J-3877 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project Number 1128. Supparted in part by a grant from the American Dairy Association. Presented at the 1960 A.D.S.A. meeting, Logan, Utah.

is an artifact. Recently, a new method for determining the trisaturated glycerides in fats has been developed in our laboratory (8). It is based on the reaction of mercaptoacetic acid with the double bonds in a fat rather than permanganate, and it appears to give considerably more reliable determinations of the trisaturated glycerides. Therefore, a number of milk fats have been analyzed by this new method, the amount of trisaturated glyceride has been compared with that calculated by random distribution, and the fatty acid composition of the trisaturated glyceride has been compared with that of the whole fat. The relation between the melting point of a fat and the melting point and proportion of trisaturated glyceride which was noticed by Kartha (14) has been tested for milk fat.

EXPERIMENTAL PROCEDURE

Milk fat samples were obtained by centrifuging melted butter and filtering the fat layer through sodium sulfate. The samples from individual cows were obtained from the University dairy farm in August, from animals on a diet of alfalfa green chop, alfalfa hay, Sudan green chop, and grain. All were in mid-lactation. The other samples were obtained from commercial butters from various geographical locations.

The trisaturated glyceride was determined by the method of Eshelman *et al.* (8). Three or four cycles were performed, and the true trisaturated glycerides were estimated by extrapolating a plot of percentage of residue vs. iodine value of residue to zero iodine value. This represented an extrapolation of less than 3% on the residue axis and less than three units on the iodine value axis in all cases.

The fatty acid composition of the milk fats and trisaturated glycerides were determined by converting them to methyl esters by refluxing with methanolic ~~sodium~~ methoxide. The methyl esters were recovered by diluting the reaction mixture with five volumes of water, acidifying with concentrated hydrochloric acid, and extracting the esters with petroleum ether. The petroleum ether extract was treated with a mixture of sodium sulfate and sodium bicarbonate to remove water and any free acids. The methyl esters were analyzed by gas phase chromatography on polyester succinate at 190° C. (6). The column was 8 ft. long. Only the acids with chain lengths longer than six carbons could be determined quantitatively. The weight percentage of each ester was obtained by multiplying the peak height by its retention time and dividing the product by the sum of such products for all the components.

The melting points of the fats were determined by the A.O.C.S. official capillary tube method (1). The iodine values were determined by the Hunter-Hyde modification of the Wijs method (13). The saponification values were determined by a micro procedure developed in this laboratory. The fatty acids containing a *cis-cis* nonconjugated pentadiene group were determined by the lipoxidase method (18).

RESULTS AND DISCUSSION

Amount and composition of the trisaturated glyceride. The amount of trisaturated glyceride in the individual milk fat samples varied from 21.5 to

32.0%. The amount in the mixed herd samples varied from 29.5 to 31.6%. Table 1 shows the fatty acid composition of the various samples of milk fat and the corresponding trisaturated glycerides. All the fatty acids present varied over a considerable range. No definite tendency for any group of fatty acids to maintain a fixed percentage could be established. Besides the saturated fatty esters in Table 1, minor amounts of methyl esters with retention times corresponding to pentadecanoate, heptadecanoate, and branched-chain pentadecanoate were detected. Monounsaturated methyl esters in all even chain lengths from 10 to 18 were detected along with C_{18} polyenoates.

TABLE 1

Fatty acid composition of some milk fat samples and of the trisaturated glycerides derived from them

Source[b]		Fatty acids[a]						
		8	10	12	14	16	18	U
Minnesota	Fat	2.17	4.33	4.27	13.1	28.9	9.74	24.5
December	TSG	2.61	6.20	6.49	18.5	41.3	25.0	
Iowa 1	Fat	1.87	3.97	4.03	13.0	28.4	10.4	25.4
December	TSG	3.62	7.20	6.54	19.6	44.6	18.4	
Kentucky	Fat	2.35	4.56	4.50	12.0	23.1	11.6	28.9
December	TSG	4.46	8.78	8.61	22.7	34.5	21.0	
Iowa 2	Fat	2.58	4.46	4.10	11.8	23.4	11.4	29.3
June	TSG	4.15	7.38	7.04	20.3	41.9	19.1	
Illinois	Fat	2.42	4.95	5.22	12.9	27.3	11.2	23.1
December	TSG	4.07	7.12	6.96	21.4	40.9	19.6	
Guernsey 1	Fat	1.97	3.65	3.37	8.33	23.2	13.5	33.0
August	TSG	3.88	7.24	6.15	15.7	44.5	22.5	
Guernsey 2	Fat	2.54	4.61	4.25	12.5	26.4	10.3	26.3
August	TSG	4.38	7.16	6.89	19.8	43.0	18.8	
Holstein 1	Fat	1.72	3.61	3.68	13.1	27.0	10.5	27.4
August	TSG	3.49	6.17	5.88	20.6	43.4	20.5	
Holstein 2	Fat	2.03	3.98	3.81	12.4	26.1	10.4	28.4
August	TSG	3.14	6.73	6.53	21.2	45.2	17.2	
Holstein 3	Fat	1.41	2.70	2.46	8.72	26.1	12.8	32.9
August	TSG	2.72	5.22	4.53	16.1	44.2	27.2	

[a] The whole fats were all assumed to contain 10.4% butyric (17) and 2.6% caproic acid. The trisaturated glyceride compositions were calculated as if they contained no butyric or caproic. All values are in mol per cent.

[b] The geographical samples are from mixed herd milk fat. The breed samples are from milk fat from individuals.

If there is no selection of the fatty acids that make up the trisaturated glyceride, the relative proportions of these fatty acids in the whole fat should be the same as those in the trisaturated glyceride. That is, the mol percentage composition of the fatty acids in the trisaturated glyceride should be the same as that of the whole fat from which all the unsaturated fatty acids have been removed. If the percentages of the saturated fatty acids in the whole fat with chain lengths from eight to 18 are reapportioned to add up to 100%, the ratio of

the mol percentage of a given fatty acid calculated in this way to the mol percentage of the same fatty acid in the trisaturated glyceride should be one. The herd and individual cow data were analyzed by computing "t" 95% confidence intervals for these ratios (trisaturated glyceride to whole fat) for each of the six fatty acids. Table 2 shows that all 12 confidence intervals bracket unity.

TABLE 2

Average ratios of the mol percentages of each fatty acid in the trisaturated glyceride to the adjusted mol percentages of the same acid in the whole fat

Fatty acids	Av. Ratio ($\bar{x}$)	$s_{\bar{x}}$	t	$\bar{x} \pm ts_{\bar{x}}$
		Herds		
8	1.010	0.078	2.776	1.010 ± 0.217
10	1.000	0.049	2.776	1.000 ± 0.136
12	0.980	0.042	2.776	0.980 ± 0.117
14	0.992	0.040	2.776	0.992 ± 0.111
16	0.941	0.028	2.776	0.941 ± 0.078
18	1.164	0.113	2.776	1.164 ± 0.314
		Individual cows		
8	1.054	0.048	2.776	1.054 ± 0.133
10	1.013	0.022	2.776	1.013 ± 0.061
12	0.983	0.008	2.776	0.983 ± 0.022
14	0.983	0.015	2.776	0.983 ± 0.042
16	0.983	0.021	2.776	0.983 ± 0.058
18	1.058	0.052	2.776	1.058 ± 0.144

It is interesting that the higher variability of the herd data is reflected by $s_{\bar{x}}$'s approximately twice as large as the corresponding values for the individuals. It is also interesting that the ratios for the fatty acids with eight, ten, and 18 carbons are higher than unity for both the herd and individual data, while those for 12, 14, and 16 are less than unity, even though these deviations can not be shown to be statistically significant. This is probably because the apparatus used for the methyl ester analysis tended to give slightly low values for stearic acid in the presence of oleic acid. This gives a slightly high ratio for stearic acid, and as a result of the way the ratios are calculated, the other ratios should be depressed slightly below unity.

There is so much error in the determination of the mol percentage of unsaturated fatty acids in the whole fat by the gas chromatographic analysis that an accurate calculation of the random value for trisaturated glyceride is not possible. However, since the above data show that no selection of the fatty acids of the trisaturated glyceride on the basis of chain length occurred, one can calculate the trisaturated glyceride from the iodine value and saponification value of the fat. Thus, let D be the double bonds per mol, S the mol fraction of the saturated fatty acid, P a correction for the polyenoate content of the fat, W_u the average molecular weight of the unsaturated fatty acids present in the fat, W_f the average molecular weight of the fat, W_t the average molecular weight of the trisaturated glycerides, T_m the mol proportion of trisaturated glyceride, T_w the weight proportion of trisaturated glyceride, I the iodine value, and SV the saponification value. Then,

$$D = 2.211 \frac{I}{SV}$$

$$S = 1 - D + P$$

$$T_m = S^3 = (1 - D + P)^3$$

$$T_w = \frac{W_t}{W_f} T_m$$

$$W_f = \frac{56{,}104}{SV}$$

$$W_t = \frac{W_f - (D - P)\ W_u}{S}$$

$$T_w = \frac{W_f - (D - P)\ W_u}{W_f} (1 - D + P)^2$$

The value for W_u for milk fat is approximately 288. It was estimated that:

$$P = 0.84 \text{ (lipoxidase polyene)} + 1.5$$

This estimate was based on a comparison of values for polyenoates obtained by the lipoxidase method and the alkali isomerization method (2). The equation attempts to take into account the molecular weight and the degree of unsaturation of the polyenoates and whether they are detected by the lipoxidase method.

Table 3 shows the comparison of the found and calculated trisaturated glyceride. The agreement is very close, and it appears that the trisaturated glyceride of both individual samples and mixed herd samples of milk fat is very close to that predicted by random distribution. There is a tendency for the experimental values to be slightly less than the calculated values, especially for mixed herd samples. However, this may be due to a slight error in the constants assumed in the equation for the calculated values.

TABLE 3

Comparison of the calculated and determined amounts of trisaturated glyceride for milk fat samples

Source[b]	I. V.	S. V.	Lipoxidase[a] polyene	Calc. TSG[a]	Experimental[a] TSG
Minnesota	33.6	228.8	2.52	33.4	31.0
Iowa 1	32.9	225.7	2.07	33.5	30.6
Kentucky	32.4	233.5	2.40	35.7	31.6
Iowa 2	38.0	227.5	2.12	26.8	29.5
Illinois	32.5	233.3	2.33	35.6	29.5
Guernsey 1	39.9	225.6	2.31	24.3	24.0
Guernsey 2	33.9	231.2	1.97	32.8	32.0
Holstein 1	35.2	232.3	1.82	30.7	28.0
Holstein 2	35.8	223.5	1.86	28.8	24.5
Holstein 3	40.9	222.9	1.98	22.4	21.5

[a] These values are expressed as weight percentages.

[b] The geographical samples are from mixed herd milk fat. The breed samples are from milk fat from individual animals.

If the milk fat really has a random amount of trisaturated glyceride in it, then the mixed herd fat should have slightly greater than the random amount of trisaturated glyceride, as calculated from the average saturated acid content of the mixed herd milk fat. This is because the cube of the average of the individual values of S is smaller than the average of the individual values of S^3. However, the deviation from random will be small compared with the experimental error.

Melting points of the trisaturated glyceride and the whole fat. Kartha (14) has reported that in many fats there is a direct relation between the amount of trisaturated glyceride and the melting point of the fat. He reported that the melting point of a fat decreased by a fixed number of degrees every time the weight percentage of the trisaturated glyceride was halved. This was confirmed by Brooker and West (5). Kartha (16) used this relation to determine if the amount of trisaturated glyceride in a fat was random. Magnusson and Hammond (19) have pointed out that the relation which Kartha found is an expression of the ideal solubility theory (4). If Kartha's relation is reduced to an equation, it states:

$$ln(T_w) = (m_t - m_f)\, k$$

where $ln(T_w)$ is the natural logarithm of the weight proportion of trisaturated glyceride, m_t is the melting point of the trisaturated glyceride in °K, and m_f is the melting point of the whole fat. The constant k in this equation is the $ln(1/2)$ divided by the constant Kartha used.

When a fat melts there is always some component which is the last to dissolve completely in the rest of the fat. The melting point and amount of this last component will determine the melting point of the fat. According to ideal solution theory, the melting point of the fat will be determined by the following equation:

$$ln(x) = (\Delta H/Rm_x m_f)\ (m_f - m_x)$$

where x is the molar proportion of the last component to dissolve and m_x is its melting point. In fats which contain trisaturated glyceride the last component to dissolve will nearly always be contained in the trisaturated glyceride, and the molar proportion of trisaturated glyceride may be substituted for x and the melting point of the trisaturated glyceride m_t may be substituted for m_x. Thus:

$$ln(T_m) = (\Delta H/Rm_t m_f)\ (m_f - m_t)$$

This equation is the same as Kartha's relation for most fats where $T_w \cong T_m$. The ratio $\Delta H/Rm_t m_f$ is $-k$, and the ratio is approximately constant over the temperature range that would be investigated.

Kartha found that for a given kind of fat, the value of k was constant. From the last equation it can be seen that this means that the heat of fusion, ΔH of the last substance to melt, must always be the same in a particular kind of fat. In Table 4 the melting point of five commercial milk fats and the melting points of the trisaturated glyceride derived from it have been used to calculate ΔH. It is apparent that for milk fat, the value of ΔH is not constant and in two cases it is unreasonably high. If the substance which is the last to dissolve

changed from sample to sample of milk fat this might explain the different values for the heat of fusion. Another possibility, which would explain the variation as well as the apparently high values for the heat of fusion, is that the particular crystal form of the last substance to dissolve is different in the fat and the trisaturated glyceride derived from it. If the latter possibility occurred, the equation would no longer be valid, and an unreasonable value for the heat of fusion could result from the calculation.

TABLE 4

Melting points of milk fats compared with the melting points of their trisaturated glyceride

Source	m. p. Fat ° C.	m. p. of TSG ° C.	Experimental[a] TSG	ΔH Kcal/mol
Minnesota	36.2	41.3	33.4	42
Iowa 1	36.2	40.6	32.7	49
Kentucky	35.4	40.7	34.1	39
Iowa 2	36.0	39.1	32.2	70
Illinois	37.1	40.4	31.9	67

[a] In mol per cent, obtained by multiplying T_m by W_f/W_t.

It is interesting that this relation which Kartha has found to hold for many types of fats does not hold for milk fat. This is possibly a reflection of the complicated structure of milk fat and the fact that no constituent of milk fat is present at a very high concentration. In such a complex mixture a slight shift in the fatty acid composition could make a change in the last glyceride to dissolve. These data also reveal the interesting possibility that the particular polymorphic form a glyceride takes may be heavily influenced by the other glycerides that are present. Thus, the last substance to melt may have one crystal form in the trisaturated glycerides and another when unsaturated glycerides are present. If the crystal form of some of the glycerides of butter are determined by the concentration in which other glycerides are present, this could be important in determining the way the consistency of milk fat varies with the fatty acid composition.

The results that have been presented have shown that the amount and composition of the trisaturated glyceride in several milk fats agree closely with the amount and composition predicted by random distribution. However, Patton *et al.* (20) and Ast and Vander Wal (3) recently have shown that the fatty acid distribution on the *alpha* and *beta* positions of the glycerol in milk fat is not random. Also, randomization of milk fat with sodium methoxide has been shown to change its physical properties (23). Vander Wal has proposed a theory of glyceride distribution, which assumes the composition of the fatty acid on the two *alpha* positions is the same but different from that on the *beta* position (22). He has shown that for milk fat this yields amounts of trisaturated glyceride which are only slightly less than that due to random distribution (3). If the amount of trisaturated glyceride that was formed was approximately random, one would also expect its fatty acid composition to be approximately random. Thus, Vander Wal's theory of glyceride structure would appear to explain all the well-established facts about the glyceride structure of milk fat.

ACKNOWLEDGMENT

We wish to thank Professor Herbert David for his statistical analysis of the data.

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SYNTHESIS OF MILK FAT IN THE BOVINE MAMMARY GLAND [1]

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SUMMARY

Milk fat synthesis was studied in normal lactating dairy cows following the intramammary infusion of C^{14}-labeled glycerol, glucose, acetate, propionate, and butyrate. The metabolite under investigation was infused into one quarter (or half) of the udder and a comparison was made of the C^{14} content of milk fat glycerol obtained from the infused and uninfused quarters. Our results indicate that glycerol is synthesized in the mammary gland from glucose but not from acetate, propionate, or butyrate. Further, we have shown that the newly formed glycerol is incorporated into milk fat. This strongly implies that one pathway of milk fat formation involves its *de novo* synthesis in the mammary gland from pools of free fatty acids and glycerol.

Milk fat consists almost entirely of mixed triglyceride in which glycerol is esterified with various combinations of at least 14 different fatty acids. It is now generally accepted that milk fat glycerol and the individual fatty acids themselves are synthesized from widely different precursors in the ruminant body, but there is little evidence to suggest where the fatty acids are esterified with glycerol to form the ultimate product, milk fat. Further, McCarthy *et al.* (8) recently proposed that two mechanisms might be involved in milk fat synthesis: alteration of pre-existing triglyceride molecules and supplemental synthesis (from nonlipid precursors).

Large quantities of glyceride are absorbed from the blood into mammary tissue (2, 7, 11). This suggests that milk fat might be formed by a fatty acid rearrangement or substitution on these preformed glyceride molecules. Indeed, a recent paper by Kumar and Lalka (4) provided direct evidence of this mechanism.

In contrast, milk fat might be assembled within the mammary gland, using glycerol and free fatty acids as building blocks. The presence of short-chain fatty acids in the mammary gland is now well established [see recent review by Luick (5)]. Intramammary synthesis of milk fat glycerol was denied by Glascock (1); however, in 1958 Wood and coworkers reported strong evidence for it (14).

This paper provides new evidence that glycerol is normally synthesized from glucose in bovine mammary tissue. We have also established that the newly formed glycerol is incorporated into milk fat. Our results almost certainly mean that milk fat is synthesized, at least in part, in the mammary gland from pools of free fatty acids and glycerol.

EXPERIMENTAL PROCEDURE

Isotope. The C^{14}-labeled substances under investigation were dissolved in 50 ml. of sterile isotonic saline and infused through a teat cannula into the

Received for publication October 19, 1960.

[1] This research was supported by a grant from the U. S. Atomic Energy Commission.

TABLE 1

Experimental details relevant to the isotope administered, site of infusion, and milk yield during the various trials

Substance infused	Millicuries Infused	Site of infusion	Milk yield (grams)			
			Milk 1		Milk 2	
			Infused quarter	Uninfused quarter	Infused quarter	Uninfused quarter
2-C^{14} glycerol	1.92	Right front	387	1,600	597	2,661
2-C^{14} glycerol	1.50	Right half	863	740	713	799
UC^{14} glucose	2.47	Right front	320	1,820	590	2,770
UC^{14} glucose	2.16	Right rear	630	1,980	856	1,835
2-C^{14} propionate	1.65	Left rear	454	590	447	729
2-C^{14} butyrate	1.37	Right front	660	1,660	460	723
2-C^{14} butyrate	1.24	Right front	306	1,550	318	1,640
2-C^{14} acetate	2.46	Right front	250	690	286	581
2-C^{14} acetate	2.77	Right front	410	1,950	360	2,360

udders of lactating dairy cows. Table 1 shows data pertaining to the metabolite studied, site of infusion, and milk yield of the infused and uninfused quarters.

Cows. The cows were milked out before infusion of the isotope and, unless otherwise noted, at 3, 12, and 24 hr. thereafter. Each milk sample was stored for 24 hr. at 4° C. to allow the cream to separate from the skimmilk. Milk fat was extracted from the cream by the Roese-Gottlieb technique. Glycerol was isolated from milk fat as the tribenzoate derivative with techniques described by Luick and Kleiber (6).

Degradation of glycerol. The distribution of C^{14} on the glycerol skeleton was studied according to a method published by Kritchevsky and Abraham (3). C-1 and C-3 atoms were obtained as the dimedon derivative following saponification with 0.6 *N* alcoholic KOH and oxidation of the glycerol moiety with $NaIO_4$ (12). Oxidation of a second glycerol sample with $Pb(OAc)_4$ yielded C-2 as CO_2 and C-1 and C-3 as formaldehyde.

Radioassay. The organic compounds were oxidized to CO_2 in a microfurnace, and $BaCO_3$ planchettes of infinite thickness were prepared for counting under a micromil G.M. detector. The results are expressed as net counts per minute and, except for the data in Table 3, have not been corrected for the eightfold dilution effect of tribenzoate C, even though this correction would be justified for samples containing high C^{14} content. Rather, the actual counting rates are listed, to prevent attaching undue significance to samples of low activity. These counting rates, when corrected for dilution, may be expressed as specific activity according to the formula: c/m $\times$ 0.047 = μc. C^{14}/gm at C.

RESULTS AND DISCUSSION

The criterion by which we decide whether a specific metabolite is used by mammary tissue for the synthesis of a given product (in this case, glycerol) rests on a comparison of the relative counting rates of that product isolated from the infused and uninfused quarters. If the metabolite is not utilized directly by mammary tissue, it would have to be taken into the general circulation and returned to the udder in a usable form before it could appear in that

TABLE 2

C^{14} in milk fat glycerol following intramammary infusion of various metabolites

Isotope infused	Milk No.	Time after infusion	C^{14} in glycerol tribenzoate: Infused quarter c/m	C^{14} in glycerol tribenzoate: Uninfused quarters c/m	Ratio infused/ uninfused
		(hr.)			
Glycerol 2-C^{14}	1	3.5	455	19	24/1
(Trial I)	2	10	355	41	9/1
	3	24	63	9	7/1
Glycerol 2-C^{14}	1	3.2	37	6	6/1
(Trial II)	2	6.0	100	36	3/1
	3	12	120	53	2/1
Glucose U-C^{14}	1	3.3	161	8	20/1
(Trial VII)	2	8.4	60	55	1/1
	3	24	42	30	1/1
Glucose U-C^{14}	1	3.3	27	9	3/1
(Trial VIII)	2	9.7	135	103	1/1
	3	24.7	58	46	1/1
Butyrate 2-C^{14}	1	3.0	3	1	3/1
(Trial VIII)	2	9.3	13	11	1/1
Butyrate 2-C^{14}	1	3.2	9	4	2/1
(Trial IX)	2	8.5	28	18	2/1
Propionate 2-C^{14}	1	4.5	6	2	3/1
(Trial VI)	2	10.3	4	18	1/4
Acetate 2-C^{14}	1	3.2	1	1	1/1
(Trial XVI)	2	8.0	12	9	1/1
Acetate 2-C^{14}	1	3.5	4	1	4/1
(Trial XVII)	2	8.5	19	12	2/1

milk product. In this case, the C^{14} content in the product taken from the uninfused quarter would be equal to that of the infused quarter. The validity of this criterion depends chiefly on the assumption that the infused metabolite can not pass from one quarter to another without first entering the general circulation. We think this assumption is justified, since there is little evidence to suggest a circulatory interchange between the various quarters (13) and, further, in similar trials with Fe^{59}- and P^{32}-labeled metabolites (Luick *et al.*, unpublished), we found no evidence that substances exchange or diffuse from one quarter to another.

TABLE 3

Distribution of C^{14} in milk fat glycerol 3.5 hr. after the intramammary infusion of 2-C^{14} glycerol (Trial 1). All values have been corrected for the diluting effect of the derivative C

Sample	Counting rate, counts/minute: ΣC	C-1 + C-3	C-2
Infused quarter	4,540	220 [a]	13,200 [c]
		218 [b]	6,900 [d]
Uninfused quarter	160	174 [a]	193 [d]
		156 [b]	150 [c]

[a] Dimedon derivative following $NaIO_4$ oxidation.

[b] Formaldehyde fraction following $Pb(OAc)_4$ oxidation.

[c] Theoretical value based on counting rate of ΣC and C-1 + C-3 according to the equation: $c/m_{(C\text{-}2)} = 3\ c/m_{(\Sigma C)} - 2\ c/m_{(C\text{-}1\ +\ C\text{-}3)}$.

[d] CO_2 fraction following $Pb(OAc)_4$ oxidation. The discrepancy between this value and the theoretical value noted for the infused quarter is presumably due to dilution with C during the oxidation procedure.

Table 2 lists the counting rates of glycerol samples taken from the infused and uninfused quarters following intramammary infusion of C^{14}-labeled glycerol, glucose, butyrate, propionate, and acetate. These metabolites have been listed in order of decreasing maximum count rate of glycerol taken from the infused quarters (and interestingly, decreasing ratio of infused quarter to uninfused quarter). Thus, comparison can be made between the different metabolites, e.g., glucose vs. acetate, in their ability to label glycerol. However, this comparison must be viewed with caution, since we have no information relevant to the extent of absorption of these substances into the udder.

Intramammary fixation of glycerol. The high labeling of milk fat glycerol taken from the quarter infused with 2-C^{14} glycerol is of particular interest, since it indicates that glycerol—if present as such in the mammary gland—may be incorporated into milk fat. In this regard, our results confirm a similar observation by Wood *et al.* (15) following injection of 1,3-C^{13} glycerol into the pudic artery of a lactating cow. In each of our trials, however, the high C^{14} content of glycerol might have occurred fortuitously via processes that did not require a net synthesis of milk fat.

First, it seemed remotely possible that the infused glycerol might have exchanged with the nonradioactive glycerol moiety of milk fat. We tested this possibility by injecting 380 μc. of 2-C^{14} glycerol into 1 liter of freshly drawn milk. This sample was then divided into two 500-ml. portions, and milk fat was extracted from each portion by techniques similar to those used in the cow trials. Glycerol was isolated from the milk fat as tribenzoate and radioassayed for C^{14} content. These derivatives gave less than three net counts per minute above background. We concluded that glyceride glycerol does not exchange in vitro with nonesterified glycerol.

Secondly, we were concerned that the infused C^{14}-labeled glycerol might have been degraded into metabolically active fragments that could, in turn, label glycerol through exchange relations. This degradation and resynthesis could occur within mammary tissue or through microbial activity during sample preparation. Regardless of cause, however, it is almost certain that the final result wauld be a significant translocation of the C^{14} label from the C-2 position to the C-1 and C-3 positions. Accordingly, a study was made of the distribution of C^{14} in milk fat glycerol (Trial 1). The results are in Table 3, where it may be seen that most of the C^{14} in glycerol from the infused quarter was on the C-2 position. This labeling pattern is almost identical with that of the infused 2-C^{14} glycerol. We, therefore, conclude that the high radioactivity found in milk fat glycerol means that glycerol is normally incorporated into milk fat in the mammary gland provided a supply of glycerol is available for this purpose.[2]

[2] Small but significant amounts of C^{14} on positions C-1 and C-3 do not necessarily mean that the infused glycerol was catabolized in mammary tissue. In fact, the even distribution of C^{14} on the C skeleton of glycerol taken from the uninfused quarter, and the similarity in count rate between these C atoms and the C-1 and C-3 positions from the infused side, indicate considerable catabolism of the infused material subsequent to its absorption from the mammary gland.

Intramammary synthesis of milk fat glycerol. Table 2 also lists the relative counting rates of glycerol samples obtained after intramammary infusion of glucose, acetate, propionate, and butyrate. These data show that in the lactating cow, only glucose leads to a significant labeling of glycerol, confirming an earlier report by Wood and coworkers (14), who injected 2, 6-C^{14} glucose into surgically prepared cows. Similarly, it refutes Glascock's conclusion, following a review of udder perfusion and mammary-slice experiments that the ". . . mammary gland is not the site of production of glycerol." The failure to demonstrate glycerol synthesis in the perfused udder (and in the mammary slice) seems understandable in light of recent criticisms of this technique (10, 14).

A considerable difference is noted in the extent of C^{14} incorporation into milk fat glycerol in Trials Glucose VII and Glucose VIII. This is particularly evident in the first milk samples taken from the infused quarters. The relatively low C^{14} content of milk fat glycerol, Trial Glucose VIII, might have been caused by a failure to completely milk-out the quarter prior to the infusion. This situation would lead to a dilution of the newly synthesized milk fat and presumably could have been prevented through the use of the milk let-down hormone, oxytocin. However, we have deliberately avoided the use of oxytocin, in order to maintain the one great advantage of our experimental technique—namely, the use of intact, lactating dairy cows under quasi-normal conditions. Perhaps equally important, Shaw and Petersen (11) have reported that oxytocin interferes with milk fat synthesis by decreasing the passage of plasma lipids into mammary tissue.

The data in Table 2 also show that the second milk fat glycerol samples taken from the infused quarters (Trials Glycerol II and Glucose VIII) contain more C^{14} than similar samples taken from the first milk. This delay in appearance of peak activity in milk fat invariably occurs following intravenous injection of C^{14}-labeled metabolites and presumably reflects delays in the synthesis and secretion of newly formed milk fat. This delay occurs in the same trials where we observe the least difference in count rate between milk fat glycerol samples taken from the infused and uninfused quarters. This suggests a diminished rate of milk fat synthesis in the mammary gland during the first 3 hr. of the trials as an alternative explanation for the inconsistencies noted in the Glucose and Glycerol trials.

Origin of milk fat. The establishment of mammary synthesis of milk fat glycerol from glucose is of particular importance, since large quantities of glucose are absorbed into the udder from the blood during lactation (2, 9). Further, Luick and Kleiber (6) recently reported that at least 70% of milk fat glycerol is synthesized from blood glucose. They postulated that this synthesis might proceed by one (or both) of two pathways: (a) synthesis of glycerol from glucose in mammary tissues, or (b) synthesis of plasma lipid glycerol, presumably in the liver, and its subsequent absorption as glyceride glycerol into the udder. The results of our experiments and the report by Wood *et al.* (14) provide strong evidence for intramammary synthesis of glycerol from glucose and for incorporation of the newly formed glycerol into

milk fat. This means that milk fat is synthesized *de novo* within the mammary gland from pools of free fatty acids and glycerol. It does not exclude, however, the simultaneous formation of milk fat from plasma glyceride via mechanisms involving fatty acid exchange, substitution, or rearrangement within the mammary gland. Indeed, the recent observations of McCarthy *et al.* (8) may be readily explained if both mechanisms contribute to milk fat synthesis in the lactating cow.

ACKNOWLEDGMENT

The author gratefully acknowledges the assistance of Chemist James M. Lucas.

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EFFECT OF SOME ENVIRONMENTAL FACTORS ON THE MILK FAT AND SOLIDS-NOT-FAT CONTENT OF COW'S MILK[1]

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SUMMARY

Two-day composite samples of milk were collected monthly from 243 Holstein and 276 Jersey cows for the first 305 days of lactation. The milk samples were analyzed for percentage of milk fat and SNF. The effects of lactation number, calendar year, stage of lactation, and month of year were determined. Age, stage of lactation, and month of year appeared to be major sources of variation in milk fat and SNF percentage. The percentage of milk fat and SNF tended to decrease as lactation number increased. Both breeds reached a low in percentage of milk fat and SNF 60-90 days after parturition, and both increased thereafter until the end of lactation. The two breeds also decreased in percentage of these two milk constituents with age.

The increased emphasis now being placed on the solids-not-fat (SNF) and total solids portion of milk makes it essential that the dairy breeder and the producer know more about factors that cause variation in these constituents in the milk of individual cows.

The effect of certain environmental factors on the composition of milk under various conditions has been reported by other workers. Bartlett (3) and Wilcox *et al.* (15) found that as the age of the cow increases, the percentage of SNF in the milk decreases. The fat and SNF content of milk were found to reach a low the first 30-60 days of lactation and then gradually increase during the remainder of the lactation (4, 6, 9). The increase of SNF in advanced lactation became less pronounced as the cow aged; at the ninth lactation SNF was 94.5% of that of the first lactation.

Seasonal effects on the fat and SNF percentage of milk have been reported by Davis *et al.* (7), Sherman (11), and Specht *et al.* (13). They found the percentage of fat and SNF in milk to be lowest in the summer months and highest during the winter months.

Wilcox *et al.* (16) found that after removing the effects of calendar months by appropriate statistical procedures, stage of lactation proved to have a highly significant effect on SNF percentage. A characteristic lactation curve was in evidence. Solids-not-fat percentage was high shortly after parturition, dropped to a lactation low at 40-60 days, increased slowly to 6 mo., then increased rapidly to the end of the lactation. Bailey (2) concluded that changes in the SNF content of milk were due to changes in the feeding practices that occurred concurrent with the change in season.

That breeding and feeding practices have an influence on the composition

Received for publication September 28, 1959.

[1] Published with the approval of Idaho Agricultural Experiment Station as Research Paper No. 473.

of milk was pointed out in a recent detailed review of literature of the United States and Canada for the period 1900 to 1957 by Armstrong (1). He reported that the composition of milk semed to show increases in both fat and SNF over that 50-yr. period. Johnson (8) also reported that the SNF and total solids portion of milk could be increased by breeding.

EXPERIMENTAL PROCEDURE

Milk samples used in this study were collected from the University of Idaho dairy herd during the periods 1935 through 1942, and 1953 through 1958. (The study was discontinuel during World War II and renewed in 1953.) Two-day composite samples of milk were collected from each cow each month for the first 305 days of lactation. Only normal milk as determined by the Hotis test and direct microscopic count was used in this study. A total of 2,430 samples from 243 lactations was collected from Holsteins and 2,760 samples from 276 Jersey lactations. The samples were made up of aliquot proportions from each milk of the individual cow during the two-day period, and contained at least 250 cc. of milk. The samples were analyzed for percentage of milk fat by the Babcock method and percentage of TS by a gravimetric method (this method involves evaporating the water by heating a 10-g. sample at 172° F. for 24 hr. and weighing the residue), the difference between the two tests being the SNF fraction.

RESULTS AND DISCUSSION

Variation due to stage of lactation and month of year. The average monthly milk yield in pounds, the percentage of fat, SNF, and TS by month of lactation for the first 305 days of each lactation are presented in Table 1.

TABLE 1

Average yield and composition of milk by month of lactation for 243 Holstein and 276 Jersey cows

Holstein					Jersey				
Month	Av. milk	Fat	SNF	TS	Month	Av. milk	Fat	SNF	TS
	(lb.)	*(%)*	*(%)*	*(%)*		*(lb.)*	*(%)*	*(%)*	*(%)*
1	1,742	3.82	8.57	12.39	1	1,027	5.20	9.47	14.67
2	1,849	3.44	8.38	11.82	2	1,011	5.06	9.36	14.42
3	1,725	3.32	8.43	11.75	3	942	5.19	9.45	14.64
4	1,600	3.36	8.54	11.90	4	867	5.26	9.62	14.88
5	1,487	3.36	8.61	11.97	5	788	5.39	9.60	14.99
6	1,372	3.41	8.98	12.39	6	717	5.46	9.68	15.14
7	1,281	3.41	8.60	12.01	7	662	5.52	9.70	15.22
8	1,171	3.42	8.65	12.07	8	583	5.66	9.67	15.33
9	1,052	3.49	8.78	12.27	9	524	5.70	9.81	15.51
10	907	3.58	8.45	12.03	10	437	5.93	9.80	15.73

The average production for 10 mo. for the 243 Holsteins in this study was 14,186 lb. of milk with 3.46% fat, 8.59% SNF, and 12.06% TS. The average production for the 276 Jersey cows was 7,558 lb. of milk with 5.43% fat, 9.61% SNF, and 15.05% TS. The percentage of milk fat was slightly lower than that

reported by Armstrong (1) and Combs *et al.* (5), whereas the per cent SNF was higher than reported by these workers. The fat content was lowest the third month of lactation for the Holsteins and the second month for the Jerseys. Both breeds had the lowest SNF content the second month of lactation. These results are in agreement with reports of other workers (4, 6, 9, 16).

The average fat and SNF percentages by months for the two breeds is presented in Table 2. Both breeds had the lowest SNF content in April. The

TABLE 2

Effect of month on milk fat and solids-not-fat content

	Holstein			Jersey		
Month	No. cows	Fat	SNF	No. cows	Fat	SNF
		(%)	(%)		(%)	(%)
J	201	3.59	8.66	224	5.68	9.68
F	194	3.53	8.62	208	5.57	9.58
M	203	3.50	8.54	211	5.43	9.52
A	204	3.51	8.50	230	5.39	9.37
M	197	3.36	8.59	247	5.16	9.49
J	197	3.36	8.58	249	5.12	9.54
J	199	3.31	8.63	240	5.21	9.60
A	201	3.29	8.66	236	5.35	9.65
S	208	3.37	8.79	233	5.52	9.76
O	208	3.58	8.70	235	5.58	9.72
N	202	3.63	8.69	225	5.69	9.81
D	206	3.68	8.70	215	5.73	9.69

Holsteins had the highest SNF content in September and the Jerseys in November. It is not known why the SNF content was lowest in April. The cows received the same ration they had been receiving the previous months. The temperature was only slightly higher than March and not as high as May.

Analysis of variance of disproportionate subgroups was calculated for stage of lactation and month effect for fat and SNF content for each breed. The source of variation, approximate significance of mean squares, and estimates of variance percentages are presented in Table 3.

Both month and stage of lactation were significant at the 1% level of probability for SNF for both breeds. It is, therefore, obvious that both month and

TABLE 3

Variance and variance percentage for month and stage of lactation effects for milk fat and solids-not-fat

	Holstein					Jersey				
Source of variation	df	Fat V	%	SNF V	%	df	Fat V	%	SNF V	%
Month	11	4.631**	27	1.003**	11	11	2.061**	11	1.981**	21
Stage of lactation	9	10.593**	62	7.726**	78	9	11.962**	66	6.970**	74
Interaction	99	1.723**	10	0.935**	9	99	3.649**	20	0.055	1
Residual	2,311	0.053	1	0.160	2	2,640	0.482	3	0.380	4

** Significant at 1% level.

stage of lactation are real effects of appreciable size. The variance estimate for months for SNF was greater in the Jersey data than in the Holstein data.

The variance of stage of lactation for SNF was greater for Holsteins than for Jerseys. Breed differences were noted by Davis *et al.* (6), who found that the range of serum solids percentage during the lactation was 0.65% for Holsteins and 0.35% for Jerseys.

Residual variation was greater among Jersey SNF estimates. The residual was composed of various random effects, of which variation among individuals would be the largest. It was assumed in this investigation, as in each of the investigations previously reported by other workers, that differences among animals would not be confounded with stage-of-lactation and month-of-year effects in such a way as to introduce any important bias. As pointed out by Wilcox *et al.* (17), such a bias would have been possible had cows with unusually high or low SNF percentages happened to fall in subclasses with very small numbers, but subclasses with small numbers did not seem to be out of line with subclasses with larger numbers in this study. In addition, since the average calving interval in this herd was about 14 mo., many cows in a given stage of lactation would not be represented in the same month of the year in successive lactations.

Month and stage of lactation were significant at the 1% level of probability for fat for both breeds. The variance estimate for months and stage of lactation for fat was higher than the variance estimate for SNF for both breeds. This would indicate that there is less variation in SNF than in milk fat due to month and stage of lactation. The variance estimate for months for milk fat was greater in Holsteins than Jerseys, whereas for stage of lactation Jerseys showed the greater variation. These results were the opposite of those found for SNF for the two breeds. Richardson and Folger (10) concluded that the relationship between fat and SNF in both normal and abnormal milk does not appear to be linear except within restricted limits of fat percentage.

Effects of age. The mean fat and SNF contents of 243 Holstein and 276 Jersey records by lactation number are shown in Table 4. The averages were based on monthly milk samples taken in the first 305 days of each lactation.

Records were not adjusted for environmental effects. Since the calving

TABLE 4

Effect of age on milk fat and solids-not-fat lactation average

Lactation No.	Holstein			Jersey		
	Records	305-Day fat	305-Day SNF	Records	305-Day fat	305-Day SNF
	(No.)	*(%)*	*(%)*	*(No.)*	*(%)*	*(%)*
1	81	3.39	8.63	89	5.42	9.47
2	52	3.40	8.60	67	5.30	9.49
3	38	3.45	8.49	43	5.34	9.55
4	30	3.43	8.44	32	5.06	9.51
5	13	3.36	8.54	17	5.04	9.44
6	13	3.40	8.44	16	5.15	9.32
7+	16	3.26	8.37	12	5.06	9.26

interval for the herd was 14 mo., stage-of-lactation and pregnancy effects should have been similar in consecutive lactations. A gradual, though irregular, decrease of both fat and SNF content with age was noted for both breeds. The slight increase in fat content in the third lactation could be the result of culling cows with low fat content after the second lactation. The gradual lowering of SNF content with age was in agreement with other investigations reported.

Effect of year. Annual means for fat and SNF percentages from samples taken monthly for the years 1935-1941 and 1953-1957 are presented in Table 5. The records were not adjusted for age, because a constant ratio of young and aged animals was maintained in this herd.

An analysis of variance of monthly tests for each cow showed significant difference among years for both fat and SNF for both breeds. A major portion of this variation for fat in the Holsteins was due to the low tests in 1936, 1937, and 1938, and the high test from 1953 through 1957. The fat tests for these two periods were significantly different. The increase in fat content from 1938 to 1954 was primarily the result of selection. A major portion of the selection pressure was devoted to increasing the fat content of the herd. After 1954, the fat content was considered to be less important than before, and the selection pressure was decreased. Hence, the decrease in fat content since 1954.

The low SNF content for 1938 and the high SNF content for 1957 account for a major portion of the variation of SNF in the Holsteins. Since SNF content was not used as a criterion for selection, the SNF content was vey irregular for the period studied.

A major portion of the variation of fat in the Jerseys was due to the low tests in 1938, 1940, and 1941, compared to high tests in 1955 and 1956. The fat tests for these two periods were significantly different. The change in fat content for the period of study was very irregular. This was to be expected, since fat content was not used in selection except when an animal tested below 5.00%.

TABLE 5

Mean milk fat and solids-not-fat content of samples from 12 calendar years

Year	Holstein				Jersey			
	Cows	Samples	Mean fat	Mean SNF	Cows	Samples	Mean fat	Mean SNF
	(No.)	*(No.)*	*(%)*	*(%)*	*(No.)*	*(No.)*	*(%)*	*(%)*
1935	9	90	3.30	8.57	25	250	5.47	9.23
1936	10	100	3.21	8.47	24	240	5.49	9.58
1937	20	200	3.18	8.47	20	200	5.38	9.65
1938	20	200	3.14	8.33	21	210	5.23	9.65
1939	24	240	3.39	8.71	17	170	5.48	9.84
1940	31	310	3.31	8.63	21	210	5.27	9.72
1941	23	230	3.42	8.64	23	230	5.25	9.73
1953	18	180	3.61	8.56	27	270	5.46	9.42
1954	20	200	3.82	8.69	26	260	5.50	9.53
1955	24	240	3.79	8.59	37	370	5.59	9.62
1956	25	250	3.57	8.49	13	130	5.68	9.53
1957	19	190	3.62	8.95	22	220	5.44	10.02

Years differing 0.29 for milk fat and 0.31 for SNF are significant at 0.05 for Holsteins.
Years differing 0.27 for milk fat and 0.29 for SNF are significant at 0.05 for Jerseys.

The SNF content varied from a low of 9.23 in 1935 to a high of 10.02 in 1957. These 2 yr. would account for a major portion of the variation, since both are significantly different from most of the other years. The changes in SNF content are very irregular for the period studied. This irregularity, too, can be explained on the basis of no selection pressure being used to change SNF content.

The results of Table 5 further substantiate the findings that the relationship between fat and SNF is not linear. For the Holsteins, the correlation coefficient between fat and SNF on individual records was 0.09, and for the Jerseys −0.02.

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EFFECTS OF FREQUENCY OF FEEDING ON PRODUCTION CHARACTERISTICS AND FEED UTILIZATION IN LACTATING DAIRY COWS

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SUMMARY

In studies with 21 lactating Guernsey cows, the effect of increased frequency of feeding on various production characteristics was reflected in trends of increasing pounds of milk production, pounds of 4% FCM, pounds and per cent of milk fat, pounds of solids-not-fat, and pounds of total solids daily per cow. Feeding interval had no effect on body weights. Increased frequency of feeding resulted in more total feed intake and a decreasing amplitude in the daily fecal chromium oxide excretion curve. When fed two, four, or seven times daily, the digestibility of dry matter was 51.59, 55.52, and 55.10%, respectively.

This study was undertaken in an effort to gain information which might serve as a basis for selecting the optimum feeding intervals for desired milk production characteristics and efficiency of feed utilization.

One of the factors which may affect feed utilization is the frequency of feeding. Annison and Lewis (1) stated that, under natural conditions, ruminants graze intermittently throughout the day and night, and marked fluctuations in rumen conditions do not occur. Tribe (14) found sheep to graze an average of six times daily between the hours of 7 A.M. and 7 P.M. Moir and Somers (10) studied the influence of the rate and method of feeding upon dry matter digestibility, rumen function, and upon the rumen microbial population. They found that both the dry matter digestibility and nitrogen retention were lower when fed the daily ration as a single feed than if the ration were fed in two or more portions during the day. Lowered average bacterial and protozoa counts were observed when the ration was fed once daily.

Gordon and Tribe (5) fed 22 ewes one and eight times daily in a single reversal experiment for 18 wk. They found a total of 196 lb. extra liveweight gain was made in the two groups when fed their ration in eight equal parts daily.

Thomas and Mochie (13) studied the effect of frequency of feeding an equalized intake on growth of dairy heifers. When fed one, two, or four times daily, the average daily gain per animal was 1.16, 1.22, and 1.85 lb., respectively. Hardison *et al.* (7) obtained similar results when they fed dairy heifers ten times daily. Mohrman *et al.* (9) found significantly greater feed consumption and gains in beef cattle when fed six times as compared to twice-daily feeding.

Limited studies are available on the effects of feeding intervals on milk production. Dawson and Kopland (3) observed that when fed twice daily,

Received for publication September 1, 1960.

Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 2211. Approved by the Director.

dairy cows produced 6% more milk and consumed 10% more hay than when fed once daily.

The effect of feeding intervals on feed utilization was studied by Gordon and Tribe (5), using sheep. They found a nitrogen balance in favor of those animals fed six times daily, as compared to one feeding daily. Moir and Somers (10) concluded that a single feeding daily resulted in poorer feed utilization than did multiple feedings. Mohrman *et al.* (9) found highly significant increases ($P < 0.01$) both in nitrogen and in energy digestibility when fed four times, as compared to once-daily feeding.

EXPERIMENTAL PROCEDURE

The data in these studies were collected from 21 lactating Guernsey cows. A 3×3 Latin-square design was used with three groups of seven cows and treatments of two, four, or seven feedings daily. There was a 2-wk. transition period which preceded the first trial and a 1-wk. transition period between each succeeding 4-wk. trial. The cows were selected on the basis of stage of lactation and milk production, with each group having four cows in the first 3 mo. and three cows in the second 3 mo. of their lactation. All cows were housed in a tie-stall barn and were allowed to go outside for exercise for 1 hr. daily between the hours of 1 and 2 P.M.

Each cow was fed 16 lb. of good-quality alfalfa hay daily. The hay to be fed at each feeding was weighed on a group basis and distributed evenly among each group at feeding time. All cows were fed 4 lb. of beet pulp daily. Concentrate was fed at the rate of 1 lb. of grain for each 4 lb. of milk produced. The amount of concentrate fed was adjusted at the beginning of each transition period which preceded each of the three trials. The balance of the daily nutrient requirements was supplied in the form of dehydrated alfalfa pellets. The basis for computing the daily nutrient requirement was 10.0% above the maximum nutrients recommended by Morrison (11) for maintenance and milk production.

Each cow's concentrate, beet pulp, and alfalfa pellets were weighed individually into a 12-qt. pail and set in front of each cow prior to each feeding. The amount fed at each feeding depended upon the feeding interval. All groups were offered the same amount of feed per day and feed weigh-backs were made on a group basis after the animals were allowed 3 hr. to consume the feed. Feed weigh-backs usually included only alfalfa hay and pellets. When there was incomplete consumption of concentrate, it was weighed and noted on the feed sheets, and fecal samples corresponding to that day's feeding were not taken. When fed two times daily the cows were fed at 2:30 A.M. and P.M.; when fed four times daily the cows were fed at 2:30 A.M., 8:30 A.M., 2:30 P.M., and 8:30 P.M.; and when fed seven times daily the cows were fed at 2:30, 5:30, 8:30, and 11:30 A.M. and 2:30, 5:30, and 8:30 P.M. All groups were milked two times daily, at 2:30 A.M. and P.M.

Feed utilization studies were conducted with each of these groups, using the chromium oxide ratio technique for determining the dry matter digestibility. Chromium oxide was mixed with concentrate feed at the rate of 10 g. of

chromium oxide per pound of concentrate, using a 300-lb. capacity horizontal feed mixer. One pound of this mixture was fed with the other feed components at the 2:30 A.M. and 2:30 P.M. feedings to all cows in each of the three groups.

Samples for the determination of digestibility were taken either rectally or by stimulation to cause feces to be voided. Samples taken for determining the diurnal excretion pattern of these cows were all taken rectally at 2-hr. intervals over a 24-hr. period. The samples from the cows in each group (7×, 4×, and 2×) were composited before determining the diurnal excretion pattern.

Samples to be used in determining the digestibility values were taken twice daily (7 A.M. and 5 P.M.), three days a week during Weeks Two, Three, and Four of each trial, respectively. Samples of approximately 50 g. each were taken from each cow and composited into a group sample of about 400 g. The samples were placed in an 8-in. Pyrex pie plate and dried in a forced-air drying oven held at 40° C. for 48 hr. At the end of the 48 hr., the samples were removed from the oven and ground through a Wiley mill with a 40-mesh screen. After grinding, the samples were thoroughly mixed and placed in separate screw-capped sample jars, labeled, and stored at room temperature until they were analyzed.

Chromium oxide determinations were made, using a slight modification of the method described by Schürch, Lloyd, and Crampton (12). A standard curve was determined, using this same procedure with known amounts of chromium oxide. Determinations for the recovery of chromium oxide mixed in a concentrate feed were also made using this method. Samples of all feed components were taken each week and composited at the end of each trial for chemical analysis. Daily milk weights were recorded for each cow and composite milk samples were taken at two consecutive milkings during the 1st and 4th wk. of each experimental period. Milk fat tests were made using the Babcock method of analysis. Specific gravity was measured at 102.0° F., using a Westphal balance standardized against distilled water at 102.0° F. Specific gravity readings were adjusted to correspond with values obtained on duplicate samples, tested under like conditions, using the Watson-Taylor lactometer. Watson-Taylor lactometer conversion tables were used to compute the total solids of the milk. The solids-not-fat were figured by difference.

Individual cow body weights were made at 1 P.M. for three consecutive days during each transition period and an average of these three days was used as the body weight value. Statistical analyses were made by the analysis of variance.

RESULTS AND DISCUSSION

A summary of the data collected from the three trials is given in Table 1.

Total milk production. The average daily milk production per cow, as shown in Table 1, when fed two, four, and seven times daily was 34.5, 37.7, and 37.0 lb., respectively. There was significantly ($P < 0.10$) more milk produced when fed four or seven times daily than when fed two times daily. Feeding 7× as compared to 4× showed no appreciable advantage in total pounds of milk produced.

TABLE 1
Mean averages for production characteristics, feed and digestibility values

Feeding frequency/day	2×	4×	7×
Total daily feed intake *(lb.)*	38.9	42.2	41.7
Four per cent FCM/lb feed *(lb.)*	0.893	0.947	0.963
Coefficient of efficiency *(%)*	28.89	30.52	30.57
Four per cent FCM *(lb.)*	35.1	40.1	40.4
Total milk *(lb.)*	34.5	37.7	37.0
Solids-not-fat *(lb.)*	3.16	3.47	3.43
Milk fat *(lb.)*	1.42	1.66	1.71
Milk fat *(%)*	4.1	4.4	4.6
Total solids *(lb.)*	4.58	5.13	5.14
Digestibility of dry matter *(%)*	51.59	55.52	55.10
Body weight *(lb.)*	1166	1176	1172

Four per cent fat-corrected milk (FCM). Table 1 shows there was little difference in 4% FCM production between the 4× (40.1 lb. daily) and 7× (40.4 lb. daily) frequency of feeding treatments, but a notable increase in 4% FCM was observed between the 2× and 4× (14.18%) and between the 2× and 7× (15.06%).

Total feed intake. The same quantity of feed was offered each group; however, there were differences in feed consumption with each respective treatment, as shown in Table 1. When fed two, four, or seven times daily, the average total feed intake was 38.9, 42.2, and 41.7 lb. per cow, respectively. There was little difference in feed intake when fed four or seven times daily, but a decrease in feed consumption that approached significance (at $P < 0.10$) was observed when fed only two times daily. The 7.20 to 8.46% increase in total feed intake when fed 7× or 4× daily as compared to 2× daily would seem to account for more than one-half of the 14.18 to 15.06% increase in 4% FCM produced when fed more frequently.

Pounds 4% FCM per pound of feed. A means of computing efficiency of feed consumption is to consider the amount of 4% FCM produced per pound of feed intake. The effects of more frequent feeding (7× and 4×) as compared to 2× in conversion of feed into 4% FCM approached the 5% level of significance. Table 1 shows a 7.84% increase in 7× (0.963) over 2× (0.893) and a 6.05% increase in 4× (0.947) over 2× (0.893) in converting feed into 4% FCM. There was only a 1.69% advantage of 7× (0.963) over 4× (0.947) in the amount of 4% FCM produced per pound of feed. These data would favor feeding at least 4× daily for optimum efficiency in converting feed into 4% FCM.

Coefficient of efficiency. Based on a formula developed by Gaines (4), coefficient of efficiency values were computed for each cow and averaged as shown in Table 1. Since Gaines's formula included a factor for body maintenance for 365 days, that factor was corrected to 28 days to fit the length of days in each respective trial. The formula used was:

$$\text{C.E.} = 52.6 \frac{\text{Fat-corrected milk}}{\text{FCM} + (0.67867\ \text{weight})}$$

When fed two, four, or seven times daily, the coefficients of efficiency were 28.89, 30.52, and 30.57, respectively. The coefficient of efficiency increase when fed

four or seven times daily approached significance (at $P < 0.10$) as compared to the twice-daily fed groups.

Pounds total solids. Table 1 shows that when fed two, four, or seven times daily, the average total solids produced per day was 4.58, 5.13, and 5.14 lb., respectively. No difference in total solids was observed when fed four or seven times daily. An increase of 12.23% in pounds of total solids was found between the 2× feeding frequency (4.58 lb.) and the 4× (5.13 lb.) or 7× (5.14 lb.) feeding frequencies. This corresponds closely to the increase in milk production when fed 2× daily as compared to 4× or 7× daily and does not reflect changes in the per cent total solids.

Pounds milk fat. The average daily pounds of milk fat produced per cow when fed 2×, 4×, or 7× daily, as shown in Table 1, were 1.42, 1.66, and 1.71 lb., respectively. There was a 3.01% increase in daily pounds of milk fat per cow when fed 7× as compared to 4× daily. A significant ($P < 0.10$) 16.90% increase in daily pounds of milk fat produced was observed between the 2× and 4× groups in favor of the more frequent feeding schedule. The milk fat percentage for the 2×, 4×, and 7× feeding frequencies averaged 4.1, 4.4, and 4.6%, respectively, as shown in Table 1. A possible explanation of the increased milk fat percentage, with increased frequency of feeding and increased milk production, could be the maintenance of levels of volatile fatty acids in the rumen which are optimal for the maximum utilization of short-chain fatty acids. This could be the result of a more uniform level of volatile fatty acid production in the rumen due to more frequent feedings.

Pounds solids-not-fat. The amount of solids-not-fat was computed by difference between the total solids and milk fat. Table 1 shows that when fed 2×, 4×, or 7× daily, the average pounds solids-not-fat per cow were 3.16, 3.47, and 3.43 lb., respectively. When fed 4× or 7×, there was no appreciable change in the pounds of solids-not-fat produced per cow daily; however, a 9.81% increase in solids-not-fat was observed between the 2× (3.16 lb.) and 4× (3.47 lb.) feeding frequencies. This was a result of more total milk being produced at the 4× feeding frequency and does not reflect a change in the per cent solids-not-fat.

Body weight. There were no significant differences in body weight found in groups when fed at different feeding intervals. Table 1 shows that when fed 2×, 4×, or 7× daily, the average body weight per cow was 1,166, 1,176, and 1,172 lb., respectively. Several workers have clearly shown that frequent feeding will increase weight gains in nonlactating ruminants [Gordon and Tribe (5) with sheep; Thomas and Mochie (13) and Hardison *et al.* (7) with dairy heifers; Mohrman *et al.* (9) with beef cattle]. The data in this experiment, however, indicated that different feeding intervals have no effect on body weight gains in lactating dairy cows.

Diurnal excretion of chromium oxide. To facilitate the taking of fecal samples at given times of the day, it was first necessary to determine the diurnal excretion pattern of chromium oxide with each respective feeding frequency. It should be emphasized that chromium oxide was fed only 2× daily, regardless

of the feeding schedule for other feed components. Fecal samples were taken rectally every 2 hr. over a 24-hr. period to establish the diurnal excretion pattern of chromium oxide. There were differences found among the 2×, 4×, and 7× feeding interval groups, as shown in Figure 1.

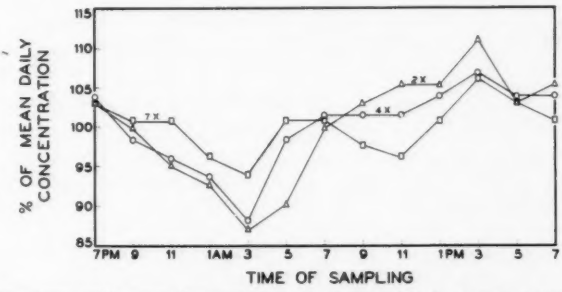

FIG. 1. Chromium oxide excretion patterns of groups fed 2, 4, and 7 times daily (100 = the daily mean excretion for all groups).

The smaller variations in the groups fed 4× and 7× daily indicate a more uniform rate of chromium oxide excretion with more frequent feed intake. It was of interest to observe that it was easiest to obtain rectal fecal samples from the group being fed 7× daily during the last 12 hr. of the 24-hr. sampling period. The fact that the groups being fed more frequently were caused to get up and eat more frequently may have affected the amplitude of these excretion curves, as those fed more frequently (4× and 7×) showed considerably less amplitude in their excretion curves than the group fed 2× daily. Balch, Reid, and Stroud (2), however, found posture to have no effect on the concentration of chromium oxide in the feces.

Sine curves fitted to the group average data are plotted in Figure 2. Equations which fitted the excretion values for each group were:

$$2\times \text{ group } E(Y) = 99.8 + 9.0 \text{ sine } 15^\circ (X - 1.5 \text{ hr.})$$
$$4\times \text{ group } E(Y) = 99.9 + 6.3 \text{ sine } 15^\circ (X - 1.3 \text{ hr.})$$
$$7\times \text{ group } E(Y) = 99.8 + 3.0 \text{ sine } 15^\circ (X - 4.1 \text{ hr.})$$

In these equations, Y is the estimated percentage of the daily mean fecal chromium oxide concentration and X is the number of hours after 7 A.M. The sine curve first crosses the mean line at 1.5 hr. (1.3 hr. for 4× and 4.1 hr. for 7×) after 7 A.M.

Although the amplitude of the curves representing the 2×, 4×, and 7× groups decreased with increasing feeding frequency, the curves followed a similar pattern, with the highest excretion of chromium oxide of the groups fed 2× and 4× being near 3 P.M. and the group fed 7× near 5 P.M. Similarly, the low values occurred near 3 A.M. in the 2× and 4× groups and near 5 A.M. in the 7× group.

Excretion patterns similar to those found in this experiment have been found by several workers, although the time of minimum and maximum chro-

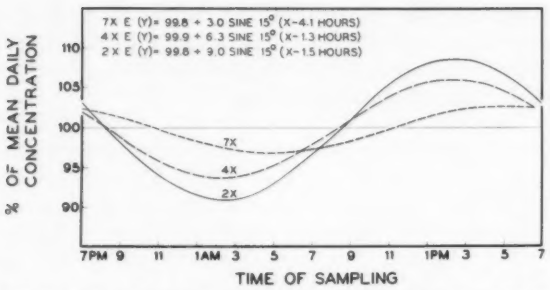
7X E (Y)= 99.8 + 3.0 SINE 15° (X-4.1 HOURS)
4X E (Y)= 99.9 + 6.3 SINE 15° (X-1.3 HOURS)
2X E (Y)= 99.8 + 9.0 SINE 15° (X-1.5 HOURS)
% OF MEAN DAILY CONCENTRATION
110
105
100
95
90
7X
4X
2X
7PM 9 11 1AM 3 5 7 9 11 1PM 3 5 7
TIME OF SAMPLING

FIG. 2. The mean excretion pattern for groups fed 2, 4, and 7 times daily fitted with sine curves where X is hours after 7 A.M. and Y is the estimated percentage of the daily mean fecal chromium oxide concentration.

mium oxide excretion varied widely. Hardison and Reid (6) concluded that the variable excretion of chromium oxide was not entirely diurnal, as the time of feed consumption and the amount of feed consumed at a given time appeared to influence the chromium oxide and time relationship. The daily variation of chromium oxide excretion in this experiment was about 10% when fed 2× daily, which closely agrees with the findings of Kameoka, Takahaski, and Morimoto (8).

In this experiment, fecal samples were taken at 7 A.M. and 5 P.M. These times were fairly close to the mean daily excretion of chromium oxide, and only small correction factors were obtained by determining the deviations of the sampling times from the mean excretion time and dividing by two. For the 2×, 4×, and 7× feeding intervals, correction factors of −1.40, −2.60, and −1.85 were computed, which show that the fecal samples were taken at times when the chromium oxide concentrations were 1.40, 2.60, and 1.85% more than the mean daily excretion.

An additional correction factor included the per cent recovery of chromium oxide in the chromium oxide concentrate feed mixture. Based on chromium oxide determinations of weighed amounts of the concentrate containing the indicator, it was possible to recover only 96.3% of the chromium oxide. This factor was applied to all chromium oxide concentration values used in the determination of dry matter digestibility.

Digestibility of dry matter. Digestibility of dry matter determinations were made on fecal samples composited for each group of cows when subjected to each feeding frequency. A minimum of three replications was made on each determination until a maximum of 2% error among sample determinations was attained. These three digestibility values were averaged and are given in Table 1.

When fed 2×, 4×, or 7× daily, the average digestibility of dry matter was 51.59, 55.52, and 55.10%, respectively. Although no differences were observed between the 4× and 7× groups, there was greater digestibility of dry matter in the groups when fed 4× or 7× daily than when fed 2× daily. An analysis of variance of the data showed these differences in dry matter digestibility when fed more frequently to approach significance (at $P < 0.10$).

The 3.93 and 3.51% higher dry matter digestibility in the groups when fed 4× or 7× daily is noteworthy and must account for a portion of the increased milk production when fed 4× or 7× daily as compared to twice-daily feeding. These data favor feeding a minimum of 4× daily, for maximum feed utilization.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Professor C. L. Gregory in the statistical analyses of the data.

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RELATIONSHIP BETWEEN RATE OF GAIN FROM BIRTH TO SIX MONTHS OF AGE AND SUBSEQUENT YIELDS OF DAIRY COWS[1]

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SUMMARY

Three breeds of experiment station cows (WSU Guernseys, Jerseys, and Holsteins, and WWES Holsteins) were used to determine if average daily gain (pounds) from birth to 6 mo. of age was related to later 305-day lactation yields of 4% fat-corrected milk (FCM). Feeding and management of the cows and calves was above-average. The 200 calves gained an average of 1.34 lb. daily and they subsequently produced an average of 10,544 lb. FCM per lactation. Comparing FCM yield by (a) simple averages or as (b) mature equivalent (ME) with average daily gain, without regard to breed, gave correlations and regressions misleadingly high. When yields were expressed in terms of (c) FCM/1,000 lb. of cow, (d) relative lactation capacity (Kleiber and Mead, J. Dairy Sci., 28:49. 1945), or (e) gross efficiency (Gaines, Science, 67: 353. 1928), gain and subsequent yield were negatively correlated. The slower-gaining WSU Jerseys produced significantly better than the faster-gaining ones as judged by any of the five measures of producing ability mentioned above. In contrast, the faster-gaining WSU Holsteins produced the best ($P < 0.05$) for FCM and mature equivalent FCM. The WSU Jerseys and WSU Holsteins were significantly different with respect to gain and subsequent production for each of the five measures [(a)-(e)] of producing ability. Similarly, WSU Guernseys and WWES Holsteins showed no significant relationships between gain and subsequent yield. On an intra-herd and intra-breed basis the linear correlation and regression coefficients between gain and yield were significant only for FCM and MEFCM and these were of a low order ($r = 0.14$ and 0.15, respectively). Judging from the results of this study, it is unlikely that gain can be used to predict producing ability.

Attempts have been made to select dairy replacement stock at an early age to avoid costs of rearing subsequently unprofitable producers of milk. If the animal should be a poor producer, more money is generally lost before a decision is made to cull it. Frequently, an adequate replacement is unavailable and the dairyman is forced to continue milking the unprofitable cow, in order to either establish or maintain his marketing base level of production. The average productive life of a dairy cow is less than 4 yr. in most dairy areas of the world. This means that a relatively few animals in each herd not only partially pay for raising many of their lower-producing herd-mates but also must return an over-all profit, if any, to the dairy operation.

Progeny testing, the best known way for evaluating transmitting ability of breeding animals, is used to predict the probable producing ability of future progeny. Though the progeny test is the superior method available, it is time-consuming, comparatively difficult to apply, and at best inefficiently used. One can theorize that appetite in the young animal might be a life-long trait and that this could be easily measured in terms of growth rate. Growth rate, in

Received for publication October 19, 1960.

[1] Scientific Paper No. 1955, Washington Agricultural Experiment Station, Project 1106.

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turn, may be correlated with milk production, since each reflects the efficiency of specialized metabolic functions which are governed by genetic and environmental factors.

Prentice (13) has reported that faster-growing heifer calves tend to produce more milk as cows. However, Davis and Willett (3), in a study of 76 animals, found no indication of a relationship between production and percentage increase in weight, wither height, and chest girth from birth to 2 yr. of age. Singh and Plum (14), using 138 animals, found no relationship between lifetime milk fat production and the chest girth or wither height at ages under 10 mo.

The chest girth gain from 10-12 mo. of age was significantly correlated ($r = 0.31$) with lifetime milk fat production on an intra-breed basis. Omitting 33 Jerseys from the original data (14), because of lack of homogeneity, similarly raised the correlation to 0.34 (12). Menge *et al.* (11) observed a correlation of 0.35 between 6-mo. weights and 90-day production in the first lactation of Holsteins.

Bonnier and Hansson (1) have shown that dairy animals have a genetically determined capacity for growth, and Brody [(2), p. 500] has shown that growth rate velocity increases to maximum by about 6 mo. of age. The purpose of this paper is to report the relationship between average daily gain from birth to 6 mo. of age and subsequent producing ability during the first one to three normal 305-day lactation records.

EXPERIMENTAL PROCEDURE

The study involved all heifer calves born after July, 1947, that completed at least one 305-day lactation by September, 1958. Jerseys, Guernseys, and Holsteins were used from the University herd in Pullman (WSU), and Holsteins from the experiment station herd located at Puyallup (WWES). Selection was involuntary during this period until at least 8 mo. of the first lactation. The animals were weighed at birth and at 6-mo. intervals thereafter. Growth rate was expressed as the average pounds/day for the first 6 mo.

Calf management. Calves were reared in individual pens for 4 to 6 mo. and then placed in group pens with other calves of similar size. Though some of the calves were used for other experiments, none of these had a significant effect on average gains from birth to 6 mo. of age, though occasionally differences occurred from birth to 3 mo. This determination was possible because individual experiments always involved a control ration of limited whole milk and hay and grain ad lib. After weaning, the feeding program was similar throughout the study period.

Cow management. At WSU, the herdsman did not change during the study period. At WWES, the cows were milked by one man, but herd supervision was changed in 1951. At both stations, feeding practices were above average. Irrigated pasture, when in season, alfalfa hay, and grass silage were fed at WWES. Alfalfa hay and alfalfa-grass silage were fed at WSU. The WSU herds were under dry-lot conditions, with the exception of the Guernseys,

during 4 to 6 wk. in late May and June of 1954-58. During this time, some pasturing was done but alfalfa hay was also available ad lib. A 14% crude protein grain mix was fed according to the production of the cow and quality of roughage available. The general feeding management was to achieve maximum production. Grain to fat-corrected milk ratios were generally wider than 1:4 at both stations, since roughage quality was nearly always above average.

Production records. Daily milk weights were used along with weekly composites for percentage butterfat at WWES and a 24-hr. composite each month and daily milk weights at WSU. Cows were milked three times daily until December, 1952, at WSU and these were adjusted to a two-time daily equivalent (9). Animals with records of less than 305 days in length because of injury, an occasional early rebreeding, or disposal, were adjusted to 305 days (5). Short records due to an animal's inability to produce, where no obvious illness or disease was observed, were not adjusted to 305 days. Lactation records on cows affected by mastitis or other obvious illnesses were excluded. If available, a maximum of three normal records was averaged and used as a cow's probable producing ability.

The National Dairy Herd Improvement Association factors (9) were used for age correction (ME). Yield was also expressed as 4% fat-corrected milk and will hereafter be called FCM. Lactation efficiency was estimated from the formula proposed by Gaines (6): Efficiency = 100 (0.172) (FCM in 305 days)/(2.42) (W) + (FCM in 305 days) (0.327). The factor, 0.172, describes the energy in milk in terms of total digestible nutrients (TDN) and is equivalent to 344 calories; 2.42 is the pounds of TDN required to maintain 1 lb. of cow for 305 days. This 2.42 lb. will, of course, be too low if the cows are overfed.

Relative lactation capacity was computed according to Kleiber and Mead (10). Their procedure converts cows of different body size to an equivalent metabolic size, using $1{,}000 + (W - 1{,}000)^{0.75}$, where 1,000 is the average weight used to express relative lactation capacity and $(W - 1{,}000)^{0.75}$ is the 0.75 power of actual weight (W) minus 1,000 lb. Brody (2), from his own voluminous studies on bioenergetics, was essentially in agreement, except that he suggested that the 7/10ths power of weight ($W^{0.7}$) described the relationship between body weight and milk energy yield. He reasoned that larger animals move more slowly and move less, and that this relationship can be described in terms of mass to surface area or in terms of resting maintenance requirements.

Statistical comparisons were made according to Snedecor in Chapters 6, 7, and 13 (15).

RESULTS AND DISCUSSION

General. Yields of FCM, using five methods of comparison, are shown in Table 1. The 305-day averages expressed as actual or mature equivalent (MEFCM) (9) represent the usual way of comparing cows. By either procedure the Holsteins (both herds) had significantly higher average production of FCM ($P < 0.01$) than Guernseys and Jerseys. Neither procedure describes gross efficiency (6) or dairy merit (2), since maintenance requirements increase as weight increases.

When FCM yield is expressed as an average per 1,000 lb. of cow (Table 1), WSU Guernseys were higher ($P < 0.05$) than Jerseys, and the WWES Holsteins were lower ($P < 0.01$) than WSU Holsteins. However, this method, as well as the method for describing gross efficiency (6), probably underestimates the true efficiency of the larger cow, because resting maintenance requirements increase less than unity per unit increase in weight (2). If the Brody (2) and Kleiber and Mead (10) generalizations are correct, then milk energy yield also increases less than unity as weight increases one unit. This may be partly or wholly due to proportionally less metabolic size (2) in larger animals but, in addition, may be due to less movement by larger animals (2). The magnitude of this effect may be considerable, as illustrated in Table 1.

A comparison of the relative lactation capacities (10) shows that WWES Holsteins are significantly lower and the WSU Guernseys and Holsteins are significantly higher. The greatest change was for WSU Holsteins. Their average yield of 9,621 lb. FCM per 1,000 lb. of cow increased to 10,382 lb. per 1,000 lb. of metabolic size.

Gains from birth to 6 mo. and subsequent yields. Table 1 shows that the 200 cows in the study gained 1.35 lb/day as calves during the first 6 mo. of extrauterine life. There were, as expected, significant differences between the breeds. The correlation between body weight during the lactation and FCM yield was 0.62 for the 200 cows and was 0.42 on an intra-herd basis (7). Yield of FCM increased 641 lb. as size increased 100 lb. for the 200 cows (8) and 685 lb. on an intra-herd basis (7). The 95% confidence limit for this latter estimate was from 475 to 895 lb. of FCM. The intra-herd correlation between daily gains the first 6 mo. and lactation body weight was 0.32, and for each increase of 1 lb. in daily gain FCM increased 178 lb. (8).

TABLE 1

Average performance on a herd-breed basis

Measurement		Herd—breed				
		WSU Guernseys	WSU Jerseys	WSU Holsteins	WWES Holsteins	Total
Records *(No.)*		52	95	138	108	393
Cows *(No.)*		28	50	66	56	200
FCM record/cow *(lb.)*	$\bar{X}$	9,712	8,566	12,372	10,573	10,544
	s	1,353	1,335	2,169	2,221	2,325
MEFCM record/cow *(lb.)*	$\bar{X}$	10,941	9,711	13,730	12,326	11,942
	s	1,665	1,534	2,351	2,337	2,601
FCM/1,000 lb. of cow *(lb.)*	$\bar{X}$	10,305	9,769	9,621	8,103	9,329
	s	1,183	1,604	1,495	1,617	1,669
Relative lactation capacity (10) *(lb.)*	$\bar{X}$	10,289	9,597	10,382	8,791	9,728
	s	1,179	1,497	1,664	1,733	1,660
Gross efficiency (6) *(%)*	$\bar{X}$	30.48	29.70	29.48	27.17	29.02
	s	1.51	2.28	2.26	2.06	2.65
Gain from birth to 6 mo. *(lb/day)*	$\bar{X}$	1.15	1.03	1.43	1.64	1.35
	s	0.17	0.16	0.19	0.22	0.32
Weight during lactation *(lb.)*	$\bar{X}$	949	885	1,294	1,308	1,147
	s	100	91	122	133	242

Daily gains were higher from birth to 6 mo. for calves of each of the herds than during each succeeding 6 mo. period up to 24 mo. of age (7). The gains to 6 mo. were more highly correlated with subsequent FCM yield than average daily gains from 6-12, 13-18, 19-24, or 0-24 mo. of age (7). Accordingly, Table 2 shows only comparisons described in Table 1.

Table 2 shows that the correlation and regression coefficients among cows are highly significant, except for relative lactation capacity. However, when the between-herd variances were removed the only significant relationships were between gain and FCM and MEFCM yields, and these were unimpressive. The results of the combined analysis (Table 2) show large differences attributable to herds. The regression coefficients for herds were significantly different in

TABLE 2

Covariance analysis of the linear relationships between average daily gain and the several comparisons of yield

Source of variation	d.f.	(r)	$b_{y.x}$	$s_{y.x}$	s_b
	(No.)	*(coeff.)*		*(lb.)*	
	Gain (x) vs. FCM (y)				
Guernsey	27	0.23	1,839	1,396	1,578
Jersey	49	−0.28[b]	−2,254[b]	1,294	1,097
Holstein—WSU	65	0.32[a]	3,529[a]	2,074	1,328
Holstein—WWES	55	0.11	1,138	2,227	1,352
Intra-herd	196	0.14[b]	1,364[b]	1,895	700
Total	199	0.39[a]	3,038[a]	2,144	506
	Gain (x) vs. mature-equivalent FCM (y)				
Guernsey	27	0.26	2,582	1,703	1,924
Jersey	49	−0.30[b]	−2,689[b]	1,481	1,256
Holstein—WSU	65	0.32[a]	3,847[a]	2,247	1,439
Holstein—WWES	55	0.14	1,465	2,336	1,418
Intra-herd	196	0.15[b]	1,589[b]	2,066	763
Total	199	0.43[a]	3,682[a]	2,360	557
	Gain (x) vs. FCM/1,000 lb. of cow (y)				
Guernsey	27	−0.05	−373	1,253	1,415
Jersey	49	−0.47[a]	−4,468[a]	1,431	1,214
Holstein—WSU	65	0.19	1,449	1,480	948
Holstein—WWES	55	−0.02	−143	1,632	990
Intra-herd	196	−0.06	−472	1,520	562
Total	199	−0.32[a]	−1,803[a]	1,581	374
	Gain (x) vs. relative-lactation capacity (y)				
Guernsey	27	0.00	14	1,250	1,412
Jersey	49	−0.44[a]	−3,910[a]	1,358	1,152
Holstein—WSU	65	0.22	1,885	1,636	1,048
Holstein—WWES	55	0.01	83	1,749	1,062
Intra-herd	196	−0.01	−69	1,588	587
Total	199	−0.12	−687	1,650	390
	Gain (x) vs. gross efficiency (y)				
Guernsey	27	−0.07	−0.65	1.6	1.8
Jersey	49	−0.46[a]	−6.18[a]	2.0	1.7
Holstein—WSU	65	0.21	2.45	2.2	1.4
Holstein—WWES	55	−0.01	−0.11	3.1	1.9
Intra-herd	196	−0.04	−0.47	2.4	0.9
Total	199	−0.30[a]	−2.65[a]	2.5	0.6

[a] Significant at the 1% level of probability.
[b] Significant at the 5% level of probability.

slope ($P < 0.05$) for each of the five yield comparisons. The 95% confidence limits of each of the individual herd regressions showed that the differences were between the WSU Jerseys and Holsteins. These may be largely genetic, since these two herds were primarily handled in the same location by the same personnel throughout the study. Daily gain of Jerseys was significantly negatively related to each of the five yield comparisons. Similarly, the daily gains of the Holsteins were positively related, though significantly so, only for FCM and MEFCM yields (Table 2).

DISCUSSION

This study suggests that factors contributing to early growth rate are not the same as those contributing to high production, particularly when some consideration is given to body weight during lactation. The slower-gaining Jerseys used in this study were significantly superior producers among the Jerseys. A different pattern of development may be involved, since this group of Jerseys (7), as well as a later group of relatives (4), shows a positive relationship between weight during lactation and FCM yield. Further work is needed to determine if the strong negative relationship between gain and yield of Jerseys is a breed characteristic or just a characteristic of the herd studied.

It is clear from management studies that no particular rate of growth is necessary at any time for later high production. These findings do not make invalid the assumption that metabolic capacity for early growth in a good environment might indicate subsequent metabolic capacity to produce. Even under these conditions, daily gains as evaluated on an intra-herd-breed basis (Table 2) show little practical value, even when no consideration is given to dairy merit as it relates to additional requirements for resting maintenance as size increases (2). However, the breed differences (Table 2) in this limited study are of sufficient magnitude to warrant further extensive studies on a breed rather than an intra-herd-breed basis.

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PNEUMATIC AND SEMIPNEUMATIC PLUGS FOR LARGE-DIAMETER RUMEN FISTULAS IN CATTLE [1]

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SUMMARY

Detailed fabrication procedures for large-diameter pneumatic and semipneumatic fistula-plugs are described. They were found, over an 18-mo. period, to be exceptionally durable, moderate in cost, and could be removed and replaced with little discomfort to the animal. They did not irritate the fistula, and required a minimum of attention (no fistula-plug care was required by four fistulated animals during a 4-mo. period).

A fistulation technique which produces a uniform fistula particularly suited to the plugs also is described. The fistulas thus produced, in the presence of the rubber plugs, showed little or no swelling immediately postoperative or during healing, and retained their size and shape satisfactorily during an 18-mo. period. No deaths from fistulation occurred.

Accompanying increased use of fistulated cattle for nutritional and physiological studies is a need for an inexpensive fistula-plug requiring minimum attention.

Johnson and Prescott (4) listed various types of plugs, but with little discussion of their advantages and disadvantages. They believed, however, that pneumatic plugs offer certain advantages over mechanical stoppers and cannulas, because such plugs are removed more conveniently and offer easier access to the rumen.

Cannulas of various kinds have been widely used for closing rumen fistulas (2, 3, 5, 6, 9, 10). The disadvantages of cannulas are many. They are usually rather heavy and become heavier as the lumen is filled with ingesta; the weight stretches the fistula, and the cannula eventually tips and falls out unless cared for. Cannulas are also hard and unyielding, thus tending to produce some irritation. In addition, plastic cannulas are subject to breakage, with subsequent loss of rumen ingesta. They have the one advantage of offering ready access to the rumen with no discomfort to the animal.

Stoppers, such as described by Schalk and Amadon (8), also tend to stretch the fistula, thus presenting a problem in keeping the plug in place, as well as the fact that they must be removed in order to gain access to the rumen.

Pneumatic plugs have been designed that eliminate the weight problem of cannulas and stoppers, but they are generally quite costly and subject to punctures and abrasions. Johnson and Prescott (4) described a pneumatic plug constructed from discarded automobile and truck inner tubes. These plugs can be vulcanized without special molds and are, therefore, quite inexpensive. Some tissue necrosis occurs with moderate pressures in these plugs, and main-

Received for publication September 14, 1960.

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taining equal pressure in each balloon is difficult. Otherwise, they appear to be entirely satisfactory.

Balch and Johnson (1) designed a complex two-piece pneumatic plug that is rather difficult to construct and has the disadvantage of being in two pieces. It requires little care, however, and leakage is minimal.

In an attempt to incorporate the advantages of the light, flexible pneumatic plug and eliminate its disadvantages, a semipneumatic plug was designed using some of the principles employed in Balch and Johnson's plug. A very durable pneumatic plug was also designed for use during motility and gas collection studies.

METHODS

The fistula-plugs were constructed with a vulcanizer (Figures 1 and 2) machined in the University shop from 3/8-in. boiler plate. Steel alloy allen bolts were used at the pressure points (E and F, Figure 2), since common steel bolts do not withstand the strain imposed by the heat and pressure necessary for vulcanization. Plates D, which are stainless steel, prevent the uncured rubber from flowing out of the joint produced when Collars B (Figure 2) are brought into position.

All materials were purchased from various local supply outlets. None of the rubber stock was of a special nature except, perhaps, the so-called combination rubber stock. This is sheet tire-patching material manufactured by Firestone Tire Company; it can be applied cold or by vulcanization.

Semipneumatic rumen fistula plug. (Step 1) From 1/16-in. pure gum sheet rubber are cut two circular pieces, one 11 in. and the other 8 in. in diameter. A 2½-in. circle is cut from the center of each disc, as shown in the upper part of Figure 3. Notches 5/8 in. deep are cut around the center opening so as to present a large surface area for vulcanization (Figure 3).

(Step 2) The 11-in. disc is stretched over E (Figure 2) and allowed to rest on Plate C. The 8-in. disc is then stretched over E, so that the apex of the tabs thus formed butt against the top of the tabs from the 11-in. rubber disc. This junction is thoroughly roughened, painted with rubber solvent, and finally

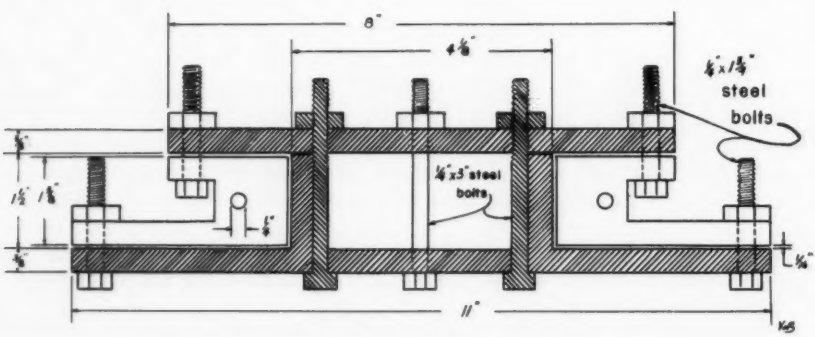

FIG. 1. Fistula-plug vulcanizer—cross section.

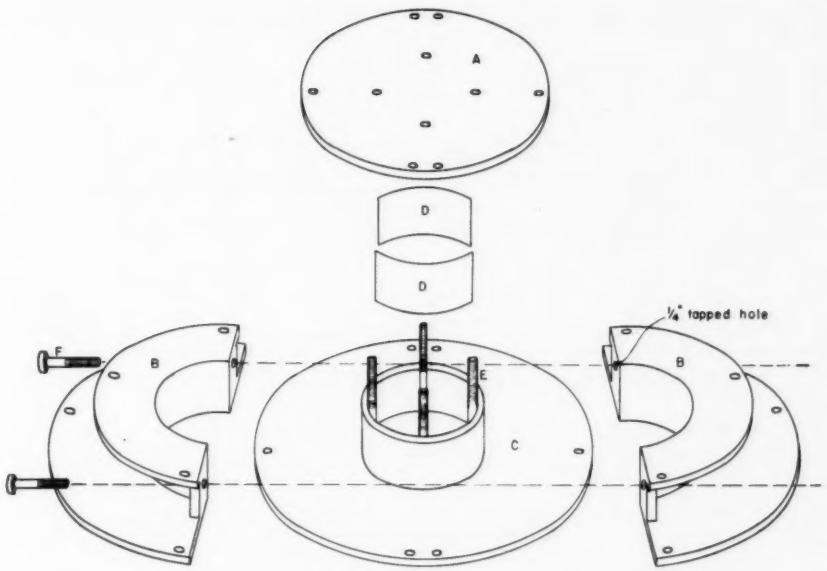

FIG. 2. Fistula-plug vulcanizer.

wrapped with a 2½-by-13-in. strip of uncured rubber (lower Figure 3). Collars B (Figure 2) are placed into position with Plates D inserted against the rubber sleeve and across the junction line formed by the leading edges of the two collars. The collars are drawn together by Bolts F (Figure 2). Plate A is next fitted over Bolts E and lightly bolted down.

Proper curing requires a uniform pressure on all surfaces of the 2½-by-13-in. band of uncured rubber; therefore, Plates A and C are clamped to Collars B with four 5-in. C-clamps.

The vulcanizer is then placed in an oven and the rubber allowed to cure for 1 hr. at 300° F.

(Step 3) The rubber sleeve thus formed is removed from the vulcanizer, roughened thoroughly, and painted with rubber solvent around its center opening. A ½-by-13-in. strip of uncured rubber is placed on the treated area around these openings (X, Figure 4). An additional ¼-by-31-in. strip of uncured rubber is placed around the 11-in. disc at Point Y (Figure 4).

(Step 4) Two pieces are cut from 1/16-in. C.I. sheet rubber, one 8 in. and the other 11 in. in diameter. (C.I. sheet rubber is sheet rubber with cloth insertion.) Each disc is roughened and treated with rubber solvent to correspond to the strips of uncured rubber on the rubber sleeve. The discs (A, Figure 4) are carefully fitted into place and Collars B (Figure 2) are again clamped into position (Plate C is reversed so that its flat side presses against the exterior of the 8-in. disc). Pressure is achieved with four 5-in. C-clamps and curing is carried out as in Step 2.

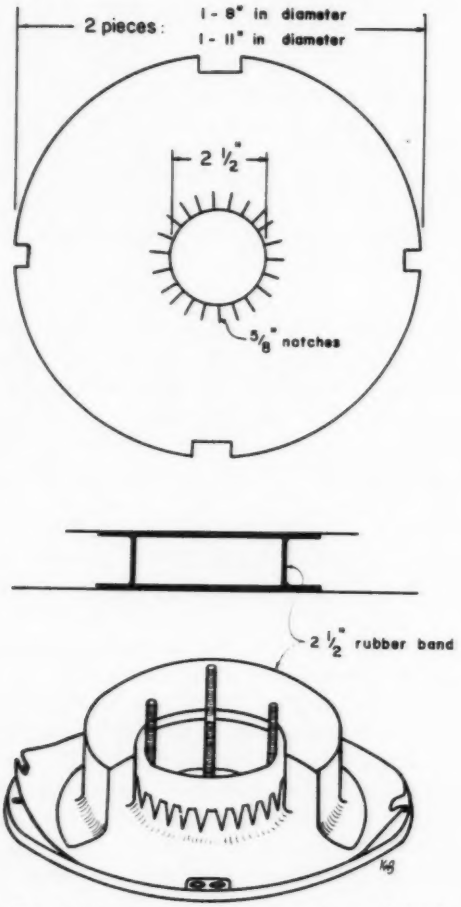

FIG. 3. Fabrication procedure for center sleeve of fistula plugs.

(Step 5) Two rings are formed from latex rubber tubing. One ring 8 in. in diameter is formed from ¾-in. I.D. latex tubing, whereas the second ring, 11 in. in diameter, is formed from ½-in. I.D. latex tubing. The rings are formed by joining the ends of the tubing together with an insertion of smaller-diameter tubing with surface roughened and treated with rubber cement. The insertion need be only 1½ to 2 in. long, and in the case of the ¾-in. tubing requires an insert of ¾-in. O.D.; similarly, an insert of ½-in. O.D. is required for the ½-in. tubing.

(Step 6) The semipneumatic plug is finished by fitting the rings between the free edges of A and B (Figure 4) and binding into place with bands 2 in. wide by 25 in., and 1½ in. wide by 33 in., respectively, for the ¾-in. and½-in. rings (C, Figure 4). The bands are cut from 1/16-in. combination rubber.

A - 1/16" C.I. sheet rubber
B - 1/16" pure gum rubber
C - 1/16" combination rubber

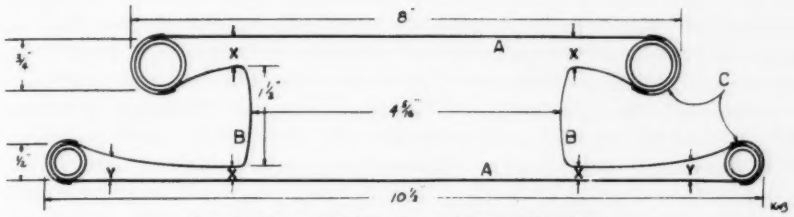

FIG. 4. Cross section of semipneumatic plug.

Pneumatic rumen-fistula plug. (Step 1) Two circular pieces, 10 in. in diameter, are cut from 1/16-in. pure gum sheet rubber and treated as in Step 1 for semipneumatic plugs.

(Step 2) Same as Step 2 for semipneumatic plugs, except that two 10-in. discs are used.

(Step 3) Two additional 10-in. discs are cut from 1/8-in. C.I. sheet (E, Figure 5, shows 3/32 in. as an actual measurement; however, it is purchased as 1/8-in. stock). A 1¾-in. hole is then cut in the center of each disc. With Side Arm B and Washer C removed, Aluminum Sleeve A (Figure 5) is placed upside down

A- Aluminum sleeve
B- Side arm 1/2" O.D.
C- Aluminum washer
D- Valve stem
E- 3/32" C.I. sheet rubber
F- 1/16" Pure gum rubber

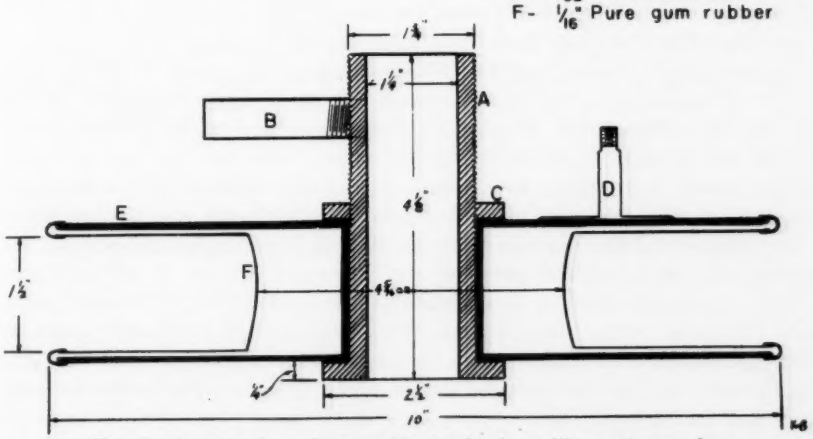

FIG. 5. Cross section of pneumatic fistula-plug with metal cannula.

over the hole in one of the rubber discs. A band of combination rubber 1½ by 6 in. is wrapped around the sleeve with the uncured side out. This produces approximately a ¾-in. lap-joint. A ⅝-in. strip of uncured rubber is then molded around the end of the rubber-covered sleeve and on to the flat 10-in. C.I. sheet disc. A 2⅛-by-6-in. strip of light tin, with ⅝-in. tabs along one edge that are bent at a right angle to the body of the tin strip, is placed around the rubber-covered sleeve and over the strip of uncured rubber. Two aircraft-type hose clamps are tightly secured around the tin cuff. A halved 4-in. steel washer with a 1⅞-in. center opening is then placed over the flared tin tabs and underlying uncured rubber. (Usually, it is helpful to place a narrow strip of uncured rubber around the opening on the opposite side of the 10-in. plate, as well, in order to produce a better union.) Plate A from the vulcanizer is placed on the opposite side of Rubber Plate E (Figure 5) and clamped to the half-washers with C-clamps. Curing is accomplished within 30 min. at 300° F.

(Step 4) Aluminum Sleeve A (Figure 5), Plate A, washer halves, tin cuff, and hose clamps are removed, and Sleeve A is then pushed, opposite to its former position, through the rubber sleeve formed in Step 3 until its top protrudes ⅛ in. above the free edge of the rubber sleeve. The second 10-in. disc of C.I. sheet is then attached to this end of the sleeve by molding a ⅝-in. strip of uncured rubber around the rubber-covered sleeve as before. From this point Step 3 is repeated.

(Step 5) With the two 10-in. discs (E, Figure 5) now attached to each other by a rubber sleeve, Sleeve F, formed in Steps 1 and 2, is placed in position as shown in Figure 5. The edges of E and F are joined together by a 1½-by-32-in. band of combination rubber. This joint is vulcanized by clamping it between Plate C and Collars B of the vulcanizer. Only one side can be vulcanized at a time, since the 8-in. surface of the vulcanizer is inadequate for the 10-in. discs. It is recommended that only one side be bound with combination rubber for the first curing period, since proper curing depends on correct temperature and sufficient pressure. When vulcanizing the second side, care must be exercised to have the valve stem open to prevent rupturing of the plug.

(Step 6) Valve Stem D (Figure 5) is applied at this point; it is 2 in. long and can be applied cold with rubber cement. Sleeve A is then pushed all of the way into the rubber sleeve, its washer and side arm are attached, and the plug is completed (Figure 5) after testing for leaks.

Fistulation. Ten animals were prepared with 4-in. ruminal fistulas according to Schnautz's technique (7), modified as follows: Feed and water were withheld on the morning of the operation; any further fasting produced a rumen that was too empty to be handled properly.

The animal was placed in a stanchion constructed to restrain lateral movement. The area of the left paralumbar fossa was shaved, thoroughly cleansed with green soap, and painted with a 2% aqueous iodine-KI solution. The surgical area was then anesthetized locally with adrocaine or xylocaine solution containing 2% epinephrine.

An area equal to the inside diameter of the sleeve of a semipneumatic plug

(approximately 3¾ in.) was marked off in the desired location. A circle of skin was then removed, as well as the underlying adipose tissue, muscle layers, and peritoneum.

Care was taken to locate the fistula far enough forward and low enough to allow both the inside and outside flaps of the plug to lie as flat as possible as well as to avoid the posterior rumen pillar.

Two loops of umbilical tape stitched superficially through the rumen wall aided in holding the rumen up while it was being sutured into place. The rumen wall was sutured to the peritoneum and muscle layers with No. 2 chromic gut as follows: A continuous stitch was used ¼ of the way, tied and so on until the entire circumference had been sutured tightly. Care must be exercised with this line of sutures, since they act as supports to the rumen wall until it becomes firmly adhered to the peritoneum.

The rumen wall was then sutured to the skin with No. 2 cotton suture, employing interrupted stitches about ½ in. apart and ¾ in. from the edge. A circle of the exposed rumen wall was then removed about ⅜ in. from the line of stitches. (The rumen in this area is well supplied with small arteries which must be properly ligated to prevent undue bleeding.) After the major bleeding had been stopped, the plug was inserted and left in place for several days before inspection.

Immediately following surgery the animal was injected with 5 g. of streptomycin sulfate; 2½-g. injections were administered daily for two or three days postoperative.

This technique produces a very uniform fistula that maintains its shape indefinitely. Care must be exercised in keeping local infection down as the fistula heals, since this can cause very severe distortion with consequential difficulties in keeping a fistula plug in place.

The animals were maintained on alfalfa hay for 3 to 4 wk. postoperative, while the fistula healed.

RESULTS AND DISCUSSION

The surgical technique described has been used over the past 18 mo. on ten mature dairy cows with excellent results. No stretching or ulceration of the fistulas is evident. Healing in the presence of the rubber plug was quite rapid, and postoperative swelling was minimal or absent. Infection occurred in only one cow, with slight distortion of the fistula; there were no deaths from surgery. This technique produces a uniform fistula that is easily maintained and easily fitted with a semipneumatic plug.

The semipneumatic plugs have been used successfully in all ten cows for 18 mo. They have been found durable and easily removable, and require minimum care. Four animals fed a hay-grain ration required no fistula plug care for 4 mo., at which time they were used in a bloat study. Only when the fistula is too large is there any danger of plug losses. (Even running and bucking seldom dislodges the plug.)

None of the plugs in use at this station have had to be discarded. However, the outer band of combination rubber tends to oxidize more rapidly than the

other parts and, consequently, has had to be replaced once or twice on a few of the plugs. This is a simple task and costs only a few cents per plug.

The pneumatic plug has been used in many gas-collection studies, motility trials, and intrarumen pressure studies, and as a permanent plug in one case, with good results. This plug, with moderate air pressure, produces an excellent gas-tight seal and has not caused tissue necrosis. Because of its low silhouette and heavy exterior plates, no punctures and abrasions have occurred. Small rumen samples are readily obtained through its metal cannula.

Most machine shops can construct the vulcanizer, but the plug construction is complex and requires some skill; however, with some practice this is readily attained.

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EFFECT OF PARTICLE SIZE ON THE DIGESTION RATE OF PURIFIED CELLULOSE BY RUMEN CELLULOLYTIC BACTERIA IN VITRO[1, 2]

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SUMMARY

Purified cellulose (approximately 100-mesh) and samples wet-ball-milled 6, 24, and 96 hr. were used as substrates for in vitro fermentations with rumen cellulolytic bacteria. Rates of digestion were compared by taking samples at 6, 12, 22, 30, and 48 hr. The data suggested that when maximum cellulose digestion is occurring, and the substrate is non-limiting, cellulose digestion of the different samples is occurring at a similar rate. The apparently faster digestion rate of the ball-milled cellulose appears to be caused by a reduction of the lag phase. Possible explanations for this are discussed. An in vitro method using calibrated large flasks, which can be sampled at various time intervals, is also described.

Baker *et al.* (2) have reported preliminary studies on the effect of particle size of purified wood cellulose on its in vitro digestibility by rumen micro-organisms. In their studies, they used three fractions which contained particles passing 40-60, 100-150, and 200-mesh screens. No differences were observed in the digestibility of the cellulose in the different fractions after a 30-hr. in vitro fermentation. These investigators also reported that the in vitro cellulose digestibility of cotton linters could be increased slightly by grinding to a fine powder in a ball-mill. Hungate (8) and others have reported that ball-milling of purified cellulose in liquid greatly reduced particle size, and have used this treatment routinely for preparing cellulose to be used as substrate in agar media for the isolation of cellulolytic bacteria.

The purified wood cellulose commonly used as substrate in this laboratory can be almost completely digested in vitro. Thus, any effect on digestion due to particle size must be either a change in the rate of digestion or a reduction in the lag phase. The present paper reports the results of studies made on the effect of particle size on the rate of digestion of purified wood cellulose.

EXPERIMENTAL PROCEDURE

Samples of rumen ingesta were obtained, through a permanent rumen fistula, from a steer maintained on a diet of mixed hay. The fluid portion of the ingesta was expelled, and a suspension of rumen bacteria was obtained by washing the pressed rumen ingesta with phosphate buffer as described by Johnson *et al.* (9). The differential centrifugation technique previously reported by Dehority *et al.* (6) was employed to obtain an inoculum consisting primarily of the cellulolytic rumen bacteria.

Received for publication October 8, 1960.

[1] Approved for publication as Journal Article No. 79-60 by the Associate Director of the Ohio Agricultural Experiment Station.

[2] The author wishes to acknowledge the technical assistance of H. W. Scott and Miss R. A. Forestal.

Fermentation flasks were incubated in a water bath thermostatically controlled at 39° C., and CO_2 was continuously bubbled through the flask contents (3). Composition of the basal medium, a modification of the medium originally proposed by Bentley *et al.* (3), is given in Table 1. The quantities

TABLE 1

Basal medium for in vitro fermentation

Component	Volume per 175 ml.
	(ml.)
Na_2CO_3 *(200 mg/ml)*	2.0
Biotin *(10 γ/ml)*	2.0
PABA *(100 γ/ml)*	0.5
Valeric acid *(5 mg/ml)*	9.0
Urea *(126 mg/ml)*	1.5
$FeCl_3 \cdot 6H_2O$ *(4.4 mg/ml)*, $CaCl_2 \cdot 2H_2O$ *(5.29 mg/ml)*	1.5
Mineral mixture [a]	35.0

[a] Ten liters of the mineral mixture contained the following in grams: Na_2HPO_4, 56.5; NaH_2PO_4, 54.5; KCl, 21.5; NaCl, 21.5; $MgSO_4 \cdot 7\ H_2O$, 5.82; and Na_2SO_4, 7.5.

reported are for the fermentations with a total volume of 175 ml., which was used for the studies reported herein. However, in some preliminary work smaller size fermentations were used and the quantity of each component was reduced in direct proportion. The pH of the fermentation media was determined periodically, and adjusted back to 6.9 with a solution of Na_2CO_3 (200 mg/ml) when required.

To study the rate of digestion, time samples had to be obtained after varying periods of fermentation. This brought up the question as to whether individual flasks, to be analyzed at the different times, or large flasks, which could be sampled at each required time, should be used. This point was investigated, using a series of 125-ml. fermentation flasks and a 250-ml. flask calibrated between volumes of 100 and 175 ml. Purified cellulose was used as the substrate. After 12, 22, 30, and 48 hr., either a small flask or a sample from the large flask was analyzed for residual cellulose by the method of Hershberger *et al.* (7). In sampling the large flasks the volume was adjusted with the calibration marks, and the flasks were gassed vigorously with CO_2 while the sample was being withdrawn. A glass tube inserted into a rubber stopper was used as a gassing tube. A large V cut in the side of the stopper allowed a sample to be withdrawn by a pipette while the contents were under CO_2. Simultaneously, the flasks were being shaken so that a uniform sample could be obtained. The results of these experiments, shown in Table 2, indicate that both methods gave similar results. The large flasks were used for the following work, because it was believed desirable to sample the same fermentation flask when possible, and because they required less space in the water baths than individual flasks.

Further work revealed that 2.4 g. of the 100-mesh cellulose substrate and an amount of inoculum equivalent to 44 ml. of the whole bacterial suspension gave a system in which the substrate was nonlimiting in 30 hr. However, after 48 hr., digestion was practically complete. This was desirable, since the digestion rate

TABLE 2

Comparison of time samples taken from individual flasks or a single large flask

Fermentation container	Time	Cellulose digested (g.)[a]	
		Cellulose added	
		1.5 g.	2.0 g.
Individual flasks	12	0.03 ± 0.03[b]	0.04 ± 0.00
	22	0.47 ± 0.01	0.46 ± 0.07
	30	0.94 ± 0.05	0.92 ± 0.05
	48	1.48 ± 0.01	1.70 ± 0.08
Large flasks	12	0.00 ± 0.00	0.00 ± 0.00
	22	0.50 ± 0.00	0.42 ± 0.01
	30	0.89 ± 0.01	0.92 ± 0.01
	48	1.49 ± 0.00	1.71 ± 0.05

[a] Each figure is the average of duplicate experiments.
[b] Standard deviation.

of the various celluloses could be compared in the first 30 hr., and the effect on total digestibility compared after 48 hr.

Three per cent suspensions of purified cellulose[3] in water were ball-milled for 6, 24, and 96 hr. to obtain substrate material of different particle size. Stones were used as a charge in the ball-mill. The cellulose suspensions and the time samples obtained after fermentation were analyzed for cellulose content by the procedure of Crampton and Maynard (5). Recovery of cellulose from the acetic–nitric acid digestion mixture was investigated because of possible solubility of the very fine material obtained by ball-milling. Four sets of duplicate samples, one digested in acid and the other not, were carried through the Crampton and Maynard analysis, and the per cent recovery of the acid-treated sample was calculated from the content of the untreated sample. For 0, 6, 24, and 96 hr. of ball-milling the per cent recoveries were 93.0, 94.4, 93.2, and 90.6, respectively.

The possibility of the fine particles obtained by ball-milling not being retained by the asbestos pad used in the Crampton-Maynard cellulose analysis was also investigated. Cellulose analysis of the 6-, 24-, and 96-hr. suspensions after ball-milling gave recoveries from the ball-mill of 102.1, 99.8, and 99.5%, respectively, based on the weight of cellulose added to the ball-mill.

RESULTS AND DISCUSSION

The photomicrographs in Figure 1 show that the particle size of the cellulose was reduced considerably by ball-milling. Crystal violet was used to stain the cellulose for these photographs. As can be seen, the length of ball-milling appears to be proportional to particle size for the 6- and 24-hr. samples; however, additional ball-milling from 24 to 96 hr. resulted in only a slight further reduction in size. For comparative purposes, the approximate average size of the cellulose particles shown in Figure 1-A is 74 μ in length and 16 μ in thickness (4). Ninety per cent of this material will pass through a 100-mesh screen.

[3] Solka Floc BW-40, Brown Company, Berlin, New Hampshire.

The rates of digestion of these different samples are shown in Figure 2. Each point on the graph represents six separate fermentation flasks. All four of the substrates were tested in each experiment, so that the averages would be comparable. The 6- and 24-hr. ball-milled samples show proportionally faster rates of cellulose digestion for the first 30 hr. However, the 96-hr. ball-milled sample did not show any marked difference in rate from the 24-hr. sample, thus substantiating the observations made earlier on the similarity of particle size for these two samples.

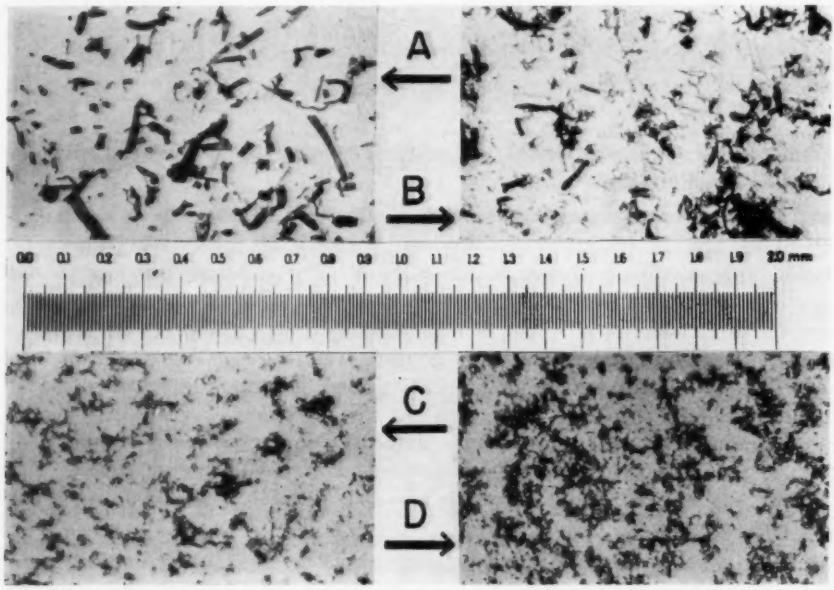

FIG. 1. Photomicrographs (×57) of purified cellulose ball-milled for varying periods of time. A—0 hr.; B—6 hr.; C—24 hr.; D—96 hr. Each small division on the scale is equivalent to 10 μ.

It is clear from these data that when the over-all rate of cellulose digestion (grams digested per hour) is calculated with the figures obtained at 22 and 30 hr., a definite difference exists. However, several points should be kept in mind: (1) the concentration of cellulose substrate which had not been ball-milled was found to be nonlimiting at 30 hr., but since the ball-milled samples had digested this much cellulose by 22 hr., their substrate level probably became limiting at that point; (2) the lag phase appears to be at least 6 hr. shorter with the ball-milled material. Thus, to determine if a true difference in rate of digestion occurs, comparisons should be made when maximum cellulose digestion is occurring, and the substrate concentration is nonlimiting. If one compares the slope of the curve for the 100-mesh cellulose between 22 and 30 hr., and for

the ball-milled samples between 12 and 22 hr., it becomes obvious that no marked differences exist.

In view of the above comments, it would appear that the difference between the 100-mesh and ball-milled cellulose can be accounted for by the reduction in apparent lag-phase. If the cellulolytic bacteria are of the fixed type, as suggested by Baker (1) and microscopic investigations carried out by the author (they must be in very close contact with the insoluble substrate before they are capable of digesting it), then the observed difference in cellulose digestion at 12 hr. (an indication of differences in lag phase) might be accounted for by surface area (available sites for attachment). The fact that this reduction in apparent lag phase appears to be in proportion to length of ball-milling would add further support to this suggestion. It is believed that the results of this study contribute basic information on the initial process in the degradation of cellulose by rumen cellulolytic bacteria.

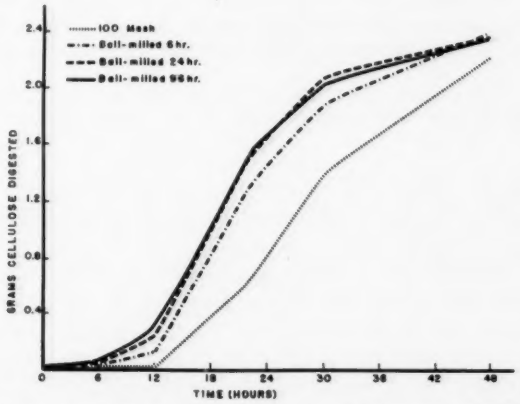

FIG. 2. Time curves of cellulose digestion by rumen bacteria, using 100-mesh cellulose ball-milled 0, 6, 24, and 96 hr. as substrate. Samples were taken at 6, 12, 22, 30, and 48 hr.

Any attempt to determine the significance of these results, with respect to the in vivo rumen fermentation, would appear to be very difficult. Since the particle sizes of the substrates used in this study are considerably smaller than those found in finely ground forage, the rapid passage of this material from the rumen to the omasum would probably cover up any increase in the rate of digestion. One might even speculate that there would be a marked decrease in the extent of digestion of these materials, because of the short time that they would remain in the rumen.

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A COMPARISON OF THE DIGESTIBILITY OF ATLAS SORGO SILAGES WITH CORN SILAGE [1, 2]

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SUMMARY

Silages made from component parts of Atlas sorgo plants were compared in digestion trials to silages made from two corn hybrids and RS 301, a sterile hybrid sorghum. Of the silages tested, Coker 911 corn silage had the most TDN. The Dixie 82 corn silage and RS 301 sorghum silage were very similar in TDN content. The removal of the heads from Atlas sorgo plants did not lower the crude protein content of the resulting silage. However, the Atlas sorgo silage in all preparations was inferior to the corn silages and the RS 301 sorghum silage as a source of both crude and digestible protein. The protein in Atlas sorgo stalks fed as silage is largely unavailable to the animal.

The addition of grain sorghum heads to Atlas sorgo plants did not improve the TDN value of the resultant silage. The energy from the extra heads from grain sorghum plants was apparently not utilized very efficiently, as they passed through the animals undigested in large quantities.

High-quality silage can be made from a sorghum which sets no seed, as shown with the RS 301. The grain in Atlas sorgo silage is apparently not essential for making good quality silage.

Corn, unlike the sorghums, depends on the grain for a large proportion of its TDN value. The coefficients of digestibility of all nutrients studied were larger for the corn silage having the greater proportion of ears.

The increased use of silage in dairy feeding programs is focusing attention on factors which influence the desirability of one plant or variety over another as a silage crop. More information is needed concerning factors that tend to limit or govern the use of plants as silage. Burrill and McCluer (3) separated corn into three portions, namely ears, stalks, and leaves and husks, and made chemical analysis of each portion. Their investigation was one of the first in which evidence was presented to show the wide differences which exist among various fields of corn in ear content of the forage at the silage-harvest stage. Later, Genter (5) reported that half the dry weight and two-thirds of the total digestible nutrients (TDN) of a productive corn plant are contained in the ear. He also observed that corn produced twice as much grain and half as much stalk as sorghum, whereas the leaf contents of these plants were similar.

The advantages of growing corn as compared to other crops for silage have been investigated by several workers (8). The amount of fat-corrected milk produced is slightly in favor of mature corn silage over immature corn silage (7). Corn silage produced more milk and more body gain than did sorghum (12), and cows consumed more corn silage than sorghum (11). Some reports have indicated advantages for Atlas sorgo silage over corn (6).

Received for publication November 3, 1960.

[1] Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

[2] Results presented in this paper are from a thesis submitted by the senior author to the faculty of the Graduate School of the University of Arkansas in partial fulfillment of the requirements for the Master of Science Degree.

Nester (9) reported that 77% of sorghum forage was stalks. The feeding value of leaf and stalk corn silage is slightly less than that of good-quality grass hay (10). Seeds made up about 28% of Atlas sorgo silage, and up to 36% of the seeds passed through the animals undigested (4). Kaffir silage seed passed through animals at a rate of 33.9%, whereas 49.5% of cane silage seed passed through animals undigested (2). Sorghum silage was only slightly less valuable than corn silage, in spite of the passage of many undigested sorghum seeds (13).

The experiments reported herein were undertaken to secure information concerning certain factors influencing the production of high-quality silage from corn and sorghum crops.

EXPERIMENTAL PROCEDURE

The forages used in these trials were grown on three different areas, fertilized according to requirement established by soil tests. Hybrid sorghum, RS 301, was harvested from one area, Atlas sorgo from another, and two corn forages from the third area.

When the Atlas sorgo heads were in the late milk stage, approximately 40% of the leaves had some deterioration due to dry weather. The area was divided into four plots. One plot was harvested as the whole plant. The second plot was harvested with all heads removed from the plants. The third plot was harvested with all heads and leaves removed from the plant, thus only the stalk and leaf sheath of the plant remained. The fourth plot was harvested as the whole plant and 200 lb. of grain sorghum heads were added per ton. The grain in the added heads was in the soft-to-hard dough stage of maturity.

Corn, Coker 911 and Dixie 81 hybrids, was planted in the third area. Both corn silages were in good condition and were harvested in the dent stages of maturity. The Coker 911 forage contained 37% ears, green weight, and the Dixie 82 contained 32% ears. Both corn forages were stored in upright concrete stave silos.

Three Holstein steers weighing 570, 668, and 700 lb., respectively, were used to determine the apparent digestibility of the silages. The steers were on a constant rate of intake throughout the trial. The steers were placed in metabolism stalls at random, the length of the stalls being adjusted by moving the feed box forward or backward until almost perfect fecal collections were possible. The total fecal collection method of determining digestibility was utilized.

During a 14-day preliminary feeding period the animals were fed all the silage they would consume twice daily at 6:00 A.M. and 5:30 P.M. Fecal collections were made morning and evening for a seven-day period on Atlas sorgo silage, Atlas sorgo silage plus heads, and Atlas sorgo silage with heads removed. The trial with Atlas sorgo stalks was terminated after ten days, with only three days of fecal collection, because of a hard freeze which spoiled a portion of the silage in the silo. With the corn silages, a six-day collection period was used because of a shortage of the silages. The feces were removed from the metal tray behind each stall as soon as possible after being voided and placed in a covered galvanized bucket. The collections were weighed twice daily, the weight recorded and sampled. The samples to be used for chemical analysis were placed

in plastic bags, frozen, and stored at −20° C. Two sealed bags were used for each sample, to prevent the loss of moisture.

A sample of silage was taken at each feeding and combined for a composite sample for analysis. The daily samples were frozen and stored at −20° C. and later combined with other samples of the same group for a composite sample of the lot of silage. Proximate analyses were determined by A.O.A.C. methods (1). Differences among digestion coefficients and values for digestible energy and total digestible nutrients were tested by the Sequential Method (14).

RESULTS AND DISCUSSION

Data pertaining to the chemical composition and digestibility of the silages are presented in Tables 1 and 2. The silage from the Coker 911 corn, Dixie 82 corn, and the RS 301 hybrid sorghum were higher in crude protein and total digestible nutrients (TDN) than were the silages prepared from Atlas sorgo. The greater crude protein content of the RS 301 silage than of the Atlas silage could have been due to the excellent green leaves at the time of harvest. Corn

TABLE 1

Chemical composition of silages
(Dry basis)

Silage	Dry matter	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Ether extract	Energy	pH
	(%)						(Cal/g)	
RS 301	27.0	5.7	9.3	29.0	49.9	6.1	4.11	4.5
Atlas (whole plant)	22.0	5.7	7.1	31.9	49.5	5.8	4.29	4.0
Atlas (heads removed)[a]	20.0	5.1	7.5	35.1	46.5	5.8	4.24	3.9
Atlas (stalks only)[b]	15.5	3.8	5.0	37.8	51.0	2.4	4.17	3.6
Atlas (heads added)[c]	22.0	4.3	8.0	29.5	51.7	6.5	4.12	3.8
Coker 911[d]	26.0	5.7	8.3	28.3	52.1	5.6	4.12	4.1
Dixie 82[e]	18.0	5.0	10.2	31.2	48.3	5.3	4.19	4.6

[a] All sorghum heads were removed.
[b] All sorghum heads and leaves were removed.
[c] Grain sorghum heads added at 200 lb/ton.
[d] Contained 36.6% ears green weight.
[e] Contained 32.0% ears green weight.

silage has been shown to have more crude protein than most sorgo silages (12). The greater proportion of ears in the Coker 911 silage was associated with lower protein content and more TDN than the Dixie 82 silage.

The removal of heads from the Atlas sorgo failed to decrease the protein content of the silage. This could be due to the small heads and poor grain set of the Atlas sorgo before harvest. The crude fiber content of the headless silage was 10% higher and of the stalk silage 19% higher than that of the whole plant silage. The stalk silage contained 0.77% crude protein on a wet basis

The addition of extra heads to the Atlas sorgo silage resulted in a silage having more crude protein than the Atlas sorgo silage from whole plants, but did not raise the TDN value as the grain equivalent would indicate.

TABLE 2

Coefficients of digestibility and total digestible nutrients of silages used

Silage	Dry matter[a]	Crude protein[b]	Crude fiber[c]	Nitrogen-free extract[d]	Ether extract[e]	Energy[f]	Total digestible nutrients (dry basis)[g]
	(%)						
RS 301	64.2	54.2	66.8	67.3	65.7	62.6	66.8
Atlas (whole plant)	58.3	36.0	61.7	61.0	55.1	56.0	59.7
Atlas (heads removed)	56.4	43.5	64.8	43.9	62.6	55.9	54.6
Atlas (stalks only)	48.7	.1	57.0	55.9	29.4	48.9	51.7
Atlas (plus heads)	51.9	39.3	55.6	57.2	64.5	50.1	58.4
Coker 911	70.9	57.6	74.4	74.4	76.7	71.5	74.2
Dixie 82	62.7	54.6	72.3	65.4	68.5	63.8	67.9

[a] Differences among means of 19.0 or greater are significant ($P < 0.05$).
[b] Differences among means of 10.7 or greater are significant ($P < 0.05$), except the difference between the value for Dixie 82 and Atlas (heads removed).
[c] Differences among means of 10.6 or greater are significant ($P < 0.05$).
[d] Differences among means of 12.0 or greater are significant ($P < 0.05$), except the difference between the values for Atlas (plus heads) and Atlas (heads removed).
[e] Differences among means of 7.5 or greater are significant ($P < 0.05$).
[f] Differences among means of 15.5 or greater are significant ($P < 0.05$).
[g] Differences among means of 12.2 or greater are significant ($P < 0.05$), except the difference between the value for Dixie 82 and Atlas (heads removed).

The digestibility of the silages used are presented in Table 2. The Coker 911 corn silage showed a higher digestibility of all nutrients for the silages tested. The digestibility of crude protein, ether extract, and N.F.E. for the Coker 911 corn silage was significantly ($P < .05$) higher than for any of the Atlas sorgo silages.

The RS 301 variety sorgo proved in this test to be almost as good as the Coker 911 and equal to the Dixie 82 corn silages in digestibility of all nutrients. This would indicate that the grain portion of the sorgo plant is not necessarily essential in the making of silage from sorgo which is equal to corn silage.

The digestibility of crude protein of the Atlas sorgo silage with leaves and heads removed proved to be lower than all other silages tested ($P < .05$). It is evident that the nitrogen of the stalk of Atlas sorgo plants is in a form which can not be readily utilized by the animal. It should be noted in this connection, however, that the digestibility is adversely affected by less than maintenance levels of protein and energy. Thus, the apparent digestibility of the protein of a feed is in part a function of the per cent of protein it carries. Ether extract, dry matter, and energy were less digestible in the stalk silage than in any of the other silages. The TDN value of the Atlas sorgo stalks is less than the TDN value of corn stover (8).

The Coker 911 had the highest digestibility of energy of the silages tested. It was well above Dixie 82 and the RS 301, which were very close in digestible energy. The energy in whole plant silage was more digestible than in the other Atlas silages. The fact that the extra heads added to the Atlas did not raise the digestible energy can be explained, in that a large amount of the seeds

from the feces showed that very little of the nutrient value of the seeds had been removed. This is in agreement with the work of Fitch and Wolberg (4). The high energy value of RS 301 hybrid sorghum, almost equal to Dixie 82 corn silage, would further substantiate the fact that the heads of the sorghum plant are hard to digest. The green leaves of the RS 301 hybrid sorgo aided in making this silage superior to the Atlas silages for energy even in the absence of grain.

The digestibility of crude fiber was significantly ($P < .05$) higher in the RS 301 variety than in the Atlas sorgo silage with heads added. The removing of the heads of Atlas sorgo tended to increase the digestion of the crude fiber. The addition of 200 lb. of grain sorghum heads depressed the digestibility of the crude fiber below that of the Atlas sorgo silage made from plants with the leaves and heads removed. The fact that the crude fiber in RS 301 was more digestible than that in Atlas with added heads ($P < 0.05$) indicates that the grain of Atlas sorgo has a depressing effect on the digestibility of crude fiber. This is further indicated in the fact that the removal of the heads from the Atlas sorgo plant increased the digestibility of the crude fiber in the resulting silage. This was not true of the grain in corn when the two corn silages of varying ear content were compared.

Coker 911 corn silage was superior to all other silages in digestibility of N.F.E. Atlas sorgo silage made from whole plants, Dixie 87 corn silage, RS 301 sorghum silage, and Coker 911 corn silage had significantly higher ($P < 0.05$) digestibility of N.F.E. than Atlas sorgo silage made from plants with the heads removed. The digestibility of the N.F.E. of Atlas sorgo silage made from plants with the heads removed was also lower than that of silage made from Atlas sorgo stalks.

The digestibility of dry matter from the Atlas silages ranged from 48.7 for Atlas sorgo silage made from stalks to 58.3 for Atlas sorgo silage made from whole plants, with the other two Atlas preparations falling in between. The RS 301 silage appeared to have a higher digestibility of dry matter than the Dixie 87 corn silage, but was below the value for Coker 911 corn silage.

The digestibility of ether extract of the Coker 911 silage was significantly ($P < .05$) higher than all other silages tested. All silages tested were significantly higher in digestibility of ether extract over the Atlas sorgo silage made from stalks. The corn silages, RS 301 variety sorgo, Atlas (plus heads), and Atlas (heads removed) had a significantly higher digestibility of ether extract ($P < 0.05$) than Atlas sorgo silage made from whole plants.

The dry matter intake was 7.5, 7.3, 4.6, and 8.2 lb. per day for the silages made from Atlas sorgo plants, headless plants, stalks, and plants with heads added, respectively. The intake of forage dry matter was 8.9, 11.1, and 9.9 lb. per day with the RS 301 sorghum, Coker 911 corn, and Dixie 82 corn silages, respectively. Thus, TDN content tended to parallel silage intake. Since the digestibility of protein was less with the Atlas sorgo silages, the addition of a portion supplement wauld probably have increased dry matter intake and improved the digestibility of the nutrients in the silages as well.

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CAUSES AND ESTIMATION OF ENVIRONMENTAL CHANGES IN A DAIRY HERD [1]

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SUMMARY

Environmental studies for 230 cows over 462 lactations were made for 7 yr. Statistical estimates of general environmental changes and the specific changes resulting from the influences of feeding and management factors were computed and compared. These estimates were based on 462 lactations of 230 cows made over a 7-yr. period in the Beltsville Holstein herd.

Studies with 293, 2 × 305 hand records indicated that the therms of estimated net energy consumed was the most important factor influencing changes in production. The length of previous dry period, season of calving, and herd size appeared to influence production, but their effects were not significant. Days carried calf was not an important effect.

General environmental changes were estimated by (a) maximum likelihood method of Henderson (6) and (b) the differences between first records of paternal half-sibs in different years, using M.E. records and M.E. records adjusted for the regression of daughter on dam. Comparisons of the methods indicated close accord between estimated changes, but adjustment of first records of paternal half-sibs for the regression of daughter on dam appeared to improve estimates of environmental changes.

Estimation of environmental fluctuations and trends by changes in factors known to influence production gave estimates agreeing closely with maximum likelihood estimates.

The data indicated that factors other than opportunity to feed, feed quality, weather, and palatabilty of the ration might have an important effect on the amount of feed consumed.

An important problem in evaluating genetic changes in the producing ability of dairy cattle involves the estimation of environmental influences. Two basic methods for estimating the effects of environment are founded on (a) general changes estimated by statistical procedures or (b) specific changes based on knowledge of factors affecting individuals or groups of cows.

The first approach adjusts records for general environmental changes by statistical means in accordance with certain criteria of classification in which the records happen to fall. The maximum likelihood method proposed by Henderson (6) yields unbiased estimates of the yearly environmental variation if the basic assumptions of the model are met (8). The maximum likelihood method was used by Harvey (4), Plowman (14), and Ormiston and Touchberry

Received for publication September 21, 1960.

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(13) to reduce the effect of yearly environmental changes when genetic trends were analyzed. The differences between the first records of paternal half-sibs in different years have also been suggested as an unbiased estimate of yearly environmental changes.

In the second approach changes in certain environmental factors are recorded and records are adjusted in accordance with the estimated effect. Several workers (2, 3, 16) have advocated this method of adjusting production records for environmental differences. Their studies indicated that level of feeding was the most important factor with herd size, season of calving, length of preceding dry period, and days carried calf of less and varying importance.

In theory, the two approaches should yield similar estimates of environmental changes between years in a dairy herd, but no research has been conducted on this relationship. The purpose of this study was to determine how well statistical estimations of environmental change agreed with certain observed changes in feeding and management.

EXPERIMENTAL PROCEDURE

Source and description of data. Data used in this study were obtained from the records of the Holstein-Friesian proved sire project of the Dairy Cattle Research Branch, USDA, Beltsville, Maryland. The present study was limited to the milk yield in 462 lactations by 230 cows initiated between September 1, 1951, and June 14, 1958. During this period continuous management efforts were made to minimize environmental variation affecting the production of cows in the project. All cows were barn-fed a standard ration based on recommendations for maintenance and production throughout all lactations. All cows were housed under similar conditions and milked two times per day. Breeding was commenced the first estrus after 80 days post-partum for cows, and the first estrus after 15 mo. of age for heifers. The change from hand to machine milking in October, 1956, was the only major management change made during the period under study.

All records were standardized to a 305-day basis by using only production in the first 10 mo. Incomplete records were standardized to a 305-day basis by factors developed from Beltsville herd data. These factors are very similar to those reported by Madden *et al.* (12).

Statistical adjustments for general changes. The maximum likelihood method as adapted by Henderson (6) to dairy cattle records was used to estimate the yearly environmental fluctuations and trends using all records made over the period of study. Groups of paternal half-sibs were used as the basic genetic groups. Since the feed supply in the Beltsville herd was similar between early October of one year and late September the following year, records were grouped for the maximum likelihood analysis into the feed year in which a major portion of the lactation was made, in an effort to maximize environmental differences between years.

Henderson (7) showed that the maximum likelihood estimates of environmental changes can be biased by the use of age-correction factors and repeat-

ability values that are not appropriate to the herd under investigation. The data used in the present study indicated the average production of first-lactation cows, after adjustment for age, was considerably lower than the mature equivalent average of later lactations. Analyses indicated these differences did not result from selection of cows on the basis of milk yield. Harvey (5) suggested a test to determine if the observed differences represented a bias in the factors used. This test was based on the report by Lush *et al.* (11), that the yield of cows making high M.E. records in first lactation, compared to their contemporaries, regresses downward toward the herd average in later lactations. Their lower-yielding contemporaries, on the other hand, regress upward toward the herd average in later lactations. If the age-correction factors were correct, the regressions of the two groups should be similar in magnitude but opposite in sign. When sire groups were divided into high and low halves on the basis of first-lactation yield, the within cow regressions of milk yield on lactation number were positive and highly significant for both halves. To eliminate any suspicion that year differences were involved, within sire and year regressions were computed: these were also positive and highly significant for all groups. The positive regressions of the cows making the higher M.E. records show that the age-correction factors used were too low for this herd. Since the regressions were approximately 4% of the mean, all first-lactation records were increased by this amount before use in other analyses.

After adjustments were made in records for the bias in the age-correction factors, the repeatability value used in this study was computed. This value was $0.58 \pm .06$, which is somewhat higher than the value generally reported (10, 11, 14).

The second estimate of general environmental changes was based on the comparison of first records of paternal half-sibs in different years. A disadvantage of this method is that, even though the animals were paternal half-sibs, important differences in the inheritance received from the dams could occur. If such differences did occur, then estimates might be biased by genetic influences. To minimize such genetic biases, adjustments were made for the regression in milk yield expected from dam to daughter. Daughter records were adjusted to a common basis within sire by the regression of daughter on dam for first-lactation milk production reported from studies of Beltsville data (9). The yearly environmental trends were estimated by the least squares method of fitting constants independently for first records adjusted for the regression of daughter on dam and the unadjusted data.

Specific adjustments for certain observed changes. Estimates of the influence of feeding level, reproductive performance, season, and herd size were obtained from the 293 completed hand records. Incomplete records and records made when cows were milked the majority of the lactation by machine were not included in this aspect of the study.

Since the general environmental changes estimated by the statistical procedures were expressed as pounds of M.E. milk, it was necessary to express specific adjustments in similar values. The measure of feeding level used,

therms of estimated net energy consumed per lactation (E.N.E.), appeared to be influenced by age. The within year regression of E.N.E. consumed on month of age was 49.3 ± 1.6. The E.N.E. consumed in individual lactations was adjusted to a mature basis by this regression prior to estimating correlations among the variables.

To estimate seasonal effects, records were grouped into two seasons within feed years by the date of calving. All records initiated between October 1 and March 31 were considered to be in Season 1, whereas records starting between April 1 and September 30 were placed in Season 2.

The number of days carried calf was used as a third variable and was considered as the number of days from the date of last service to the 305th day of lactation. The fourth variable, length of previous dry period, was computed as the number of days from last milking in the previous lactation to the date of calving of the present lactation. Only second and later lactation records were considered to have a previous dry period. Herd size was considered as the number of cows that made the majority of their records in a particular feed year.

The correlation coefficients between all six variables were calculated. The partial regression coefficients and the related statistics were then computed by inversion of the matrix of correlations. Standard errors and other statistics necessary for tests of significance were calculated from elements of the inverse matrix. The partial regression coefficients were used to estimate the differences in yearly production resulting from changes in the variables measured.

RESULTS

Statistical adjustments for general changes. The maximum likelihood estimates of yearly environmental changes, the M.E. herd averages, and the M.E. herd averages adjusted for these estimates are shown in Table 1. The general changes between years are expressed as deviations from the average environment of the period studied. Algebraic subtraction of these deviations from the

TABLE 1

M.E. herd averages (milk yield) and maximum likelihood estimates of environmental deviations by years

Year	No. of records	M.E. herd average	Estimated environmental change	M.E. herd average adjusted for estimated environmental change
			(lb.)	
1952	55	17,287	798 ± 430[a]	16,489
1953	59	16,607	322 ± 407	16,285
1954	62	17,399	676 ± 354	16,723
1955	66	16,245	−174 ± 341	16,419
1956	77	16,588	−176 ± 327	16,764
1957	79	15,950	−881 ± 358	16,831
1958	64	17,031	−565 ± 562	17,596

[a] Standard error.

M.E. herd averages should place all records on the same basis with respect to the average environment influencing M.E. milk yield. The changes estimated by the maximum likelihood procedure indicated the environment influencing M.E. milk production became less favorable in the Beltsville herd between 1951 and 1958 (Table 1).

Although the standard errors of the individual constants (Table 1) appear high, the 2% reduction in total variation resulting from adjusting by maximum likelihood was highly significant (Table 2). The within cow variance was re-

TABLE 2

Relation of variances with and without adjustments for maximum likelihood estimates of year changes

Source of variation	Degrees of freedom	Mean squares [a]	
		Without adjustment	Adjusted for year changes
Among cows	229	106,268**	107,368**
Reduction due to year adjustments	6		102,028**
Within cows	232	28,308	25,317
Repeatability ($K_o = 2.01$)		0.58	0.62

[a] Mean squares coded to nearest 100.
** = $P < .01$.

duced 11%, the between cow variance increased, and the repeatability increased from 0.58 to 0.62. Plowman (14), using milk fat records, reported within cow variance decreased and repeatability increased from 0.41 to 0.50 after adjustment for yearly environmental changes estimated by the maximum likelihood method.

The environmental changes estimated by fitting constants on a within sire basis between first records of paternal half-sibs in different years are shown in Table 3. These estimates were computed for both the unadjusted data and the data after adjustment for the regression of daughter on dam. These con-

TABLE 3

Environmental deviations estimated by the differences in first records of paternal half-sibs and the maximum likelihood

Year	No. of records [a]	First records of paternal half-sibs		Maximum likelihood
		With adjustment [b]	Without adjustment	
		(lb.)		
1952	14	803	672	798
1953	17	− 31	−171	322
1954	19	192	284	676
1955	22	−717	−785	−174
1956	25	222	569	−176
1957	20	−220	−109	−881
1958	8	−249	−460	−565

[a] Number of first records available in each year.
[b] Records adjusted for the within-sire regression of daughter on dam.

stants show trends similar to the maximum likelihood results. The changes estimated from the adjusted data appear to deviate less from the maximum likelihood results than those from the unadjusted data.

The simple correlation coefficients for all relationships among and between the specific environmental variables studied and M.E. milk yield are shown in Table 4.

TABLE 4

Correlation coefficients

	M.E. milk	Therms E.N.E. consumed	Season of calving	Days carried calf	Previous dry period
Therms E.N.E. consumed	.512**				
Season of calving	−.164*	−.092			
Days carried calf	−.127*	−.141*	.011		
Previous dry period	−.029	.266**	.083	−.072	
Herd size	−.077	.025	.166**	−.030	−.291**

* $P < .05$.
** $P < .01$.

The correlations between M.E. milk yield and the independent variables were essentially similar to those reported by other workers (1, 16). The factors responsible for the significant correlations between days carried calf and therms E.N.E. consumed, and length of previous dry period with therms E.N.E. consumed and herd size, are not clear.

The correlations were used to compute the standard partial regression coefficients and partial regression coefficients shown in Table 5. This analysis

TABLE 5

Effect of certain environmental influences on M.E. milk yield

Environmental influence	Standard partial regression	Partial regression
Therms E.N.E. consumed	.496**	1.19
Season of calving	−.105	−473.8[a]
Days carried calf	−.058	− 2.07
Length of previous dry period	.132	4.46
Herd size	−.074	− 20.19

[a] This partial regression is a least-squares estimate of the difference between the summer and winter seasons.
$R^2 = .284$.
** = $P < .01$.

indicated E.N.E. consumed was the most important individual source of variation in M.E. milk production in the Beltsville herd, and was the only partial regression statistically significant. The order of importance of the other variables was as follows: length of previous dry period, season of calving, herd size, and days carried calf.

Other workers (1, 2, 15, 16) also reported that feeding level was the most important single factor influencing milk production. The effects of length of

previous dry period, season of calving, herd size, and days carried calf were similar to other reports (1, 3, 15, 16).

The Beltsville M.E. herd averages were adjusted for yearly changes in the specific factors by a prediction equation derived from the partial regression coefficients. The estimated changes between years in M.E. milk yield resulting from changes in the combined effect of the specific factors studied are shown with the general changes estimated by the maximum likelihood analysis in

TABLE 6

Estimated differences in production for years from the individual and combined influences of five environmental factors compared with the maximum likelihood estimates of general environmental deviations

Year	Therms N.E. consumed	Season of calving	Days carried calf	Length of previous dry period	Herd size	Combined effect of specific factors	Maximum likelihood estimates of general change
1952	−570	111	0	190	211	− 58	798
1953	120	− 6	−3	173	130	414	322
1954	689	− 10	−9	− 61	70	679	676
1955	−264	− 11	2	−141	− 11	−425	−174
1956	− 56	− 11	1	− 80	−233	−379	−176
1957	−252	−151	0	− 15	−274	−692	−881

Table 6. It is evident that E.N.E. consumed, length of previous dry period, and herd size were responsible for a major share of the environmental changes. Season of calving had an important influence only in 1952 and 1957, with little variation associated with days carried calf. It is evident that a considerable portion of the general change indicated by the maximum likelihood resulted from changes in the specific factors measured.

DISCUSSION

Although the two statistical methods of estimating general environmental trends used are both based on changes in the production of the same or related cows, each has certain advantages and disadvantages. The maximum likelihood method has the advantage of utilizing all records and not being biased by selection. It may, however, be biased by improper repeatability values or age-correction factors. Age-adjustment factors which may not properly account for differences between lactations, improper repeatability values, and selection of cows with production records, are not sources of bias when first records of paternal half-sibs are used to appraise yearly environmental variation.

If the maximum likelihood procedure had been biased by repeatability values that were too high, or age-correction factors that overadjusted first records, then the estimates from the maximum likelihood would have shown a more downward trend than those computed from the paternal half-sibs. This supports the assumption that the repeatability and age-correction factors used were appropriate to the Beltsville herd. If one makes the assumption that the maximum likelihood accurately measured yearly fluctuations, it appears that

the within sire adjustment for the regression of daughter on dam reduced the genetic bias influencing the unadjusted data. When the differences between groups of paternal half-sibs are to be used to evaluate trends, such adjustments might be important.

Although it is of interest to note the fluctuations and trends in the environment of dairy herds, the real importance of estimating changes is to enable dairymen to more accurately estimate genetic changes in their cattle. If environmental trends or fluctuations are known, the confounding of environmental and genetic changes may be reduced by proper adjustments. In theory, the two basic methods should be closely related, but under certain conditions only one might be used with optimum efficiency. It is desirable, therefore, to determine the association of the environmental changes as estimated by these two methods.

The environmental trends estimated by the statistical procedures and those based on known changes in feeding and management are essentially similar. Although the reasons for the differing estimates of the first year are not apparent, it could easily have resulted from the effects of disease, weather conditions, or personnel handling the cows.

From these data it appears that variation in the therms of estimated net energy consumed, season of calving, days carried calf, length of dry period, and herd size were responsible for a considerable portion of the environmental changes between years. These results support the use of certain statistical procedures to estimate environmental fluctuations and trends in situations where detailed information on factors affecting individual cows is not available.

One of the striking points shown by the study was the difference in amount of variation indicated by the maximum likelihood analysis to result from year differences (2% of the total variation) and the 28% accounted for by the study of specific effects (Table 5). If these estimates are comparable, they imply that the within year environmental variation was at least as important as between year variation in the Beltsville herd.

Another factor possibly influencing the difference in variation accounted for is associated with the fact that although cows were fed all the roughage they would consume with a minimum of 4 lb. of grain daily, regardless of recommended levels, changes in consumption had an important influence on production. Some have considered that management factors such as feed quality, palatability, and the method of feeding are the major factors influencing the amount of feed consumed. Since all cows were treated alike and the correlations between feed consumed and other measures were small, there is some suggestion that other factors may be important influences. These other factors might be genetic in nature and, if this were true, adjustment for amount of feed consumed would not be warranted. It would appear that further study on the nature of these factors influencing feed consumption should be considered in future studies of specific environmental influences.

Certain routine changes in the feeding program during the year appear to be an effective way of classifying records. Classification of records to coincide

with normal yearly changes in feeding and management may be more sensitive for getting accurate estimates of year differences than classifying strictly on a calendar basis.

The results of this study imply that the maximum likelihood method estimates fluctuations in herd environment that are associated with specific changes in feeding and management. In turn, this implies that in situations where the basic assumptions are valid, adjustment by maximum likelihood should make estimates of genetic changes more accurate in herd and population studies.

The magnitude of the age-correction and repeatability values computed in this study illustrates that caution should be used in applying the maximum likelihood method without testing the validity with which the data meet the necessary assumptions. Even with the relatively large number of records per year available for this study, the standard errors of the yearly deviations were high and indicate serious sampling errors may result from using small numbers of records.

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COMPARISON OF PROCEDURES USED FOR EVALUATING SIRES USED IN ARTIFICIAL INSEMINATION

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SUMMARY

The correlations between evaluation procedures, based on deviations from contemporaries of first records and averages of records of daughters and a method currently used at the New York Dairy Records Processing Laboratory, are computed for records of daughters of 198 A.I. Holstein sires. These correlations are successively recalculated for number of daughters per sire greater than 20, 50, and 200. The relationships are relatively low when all sires are included in the analysis. For more than 50 daughters, a few correlations are large enough to warrant consideration of these procedures in sire evaluation when computing facilities are limited.

The importance of proving sires has increased with the growth of artificial insemination. Many procedures have been proposed for evaluating sires used in artificial insemination programs based on the records of their progeny. In fact, the progeny test appears to be the most valuable tool by which sires can be chosen for heavy service. Some of the proposed evaluating procedures are very time-consuming unless modern high-speed computing equipment is available. The purpose of this study was to compare the evaluation of sires based on a method currently used at the New York Dairy Records Processing Laboratory, with evaluation by various other methods some of which require much less computational effort.

DATA

The D.H.I.A. records used were 305-day, 2×, M.E. fat and milk production records of daughters of 198 Holstein sires used in artificial insemination. The number of records per sire ranged from one to 5,096. The number of daughters with first records per sire varied from one to 2,338. First records were defined as records of cows which freshened at less than 33 mo. of age.

EXPERIMENTAL PROCEDURE

In all studies of this type a problem is posed when an attempt is made to determine a criterion for evaluating a sire's true genetic worth. If large numbers of records are available from each sire's progeny, and if these records were made under random environmental conditions, at least random with regard to the population considered, then an average of such records would provide a very accurate measure of a sire's genetic merit. Such a situation does not exist at this time. Therefore, an evaluation procedure must be arbitrarily chosen with which to compare other procedures. If the control procedure is better than the others compared, then it is apparently a good choice. The procedure which was chosen as the control is a slight modification of one presented by

Received for publication October 31, 1960.

Henderson *et al.* (5) and is the method used at the Dairy Records Processing Laboratory at the time of this study (January, 1960). Heidhues *et al.* (2) extensively discuss the statistical basis of this procedure. Henderson (4) has shown, in general, that the selection index procedure maximizes the probability of correct ranking for breeding value. It seems reasonable to think that the control procedure, which is a slight modification of the selection index procedure, evaluated bulls according to estimated breeding value better than the other procedures examined.

For completeness this procedure is briefly described. Breeding value is estimated as:

$$\text{EBV} = \mu + \frac{m}{m+12}\left[\overline{d} - b(\overline{X} - \mu) - \mu\right]; \qquad (1)$$

where m = number of daughters of a sire,

μ = breed average of the preceding 5 yr.,

$\overline{d}$ = the mean of the averages of the records of each daughter,

b = the intra-sire regression of daughter records on adjusted stable-mate averages which has been estimated to be 0.9 from New York data by Henderson (3), and

$\overline{X}$ = the mean of the adjusted stable-mate averages associated with the daughter records.

The adjusted stable-mate average, X, is computed as

$$\frac{\text{herd season total} - \text{cows record} + \text{breed season average}}{n}$$

which equals breed season average $+ \frac{n-1}{n}$ [stable-mate average $-$ (breed season average)]; where n is the number of cows in the herd season and the stable-mate average is the average of the records made in a herd season excluding the cow's record.

The other methods were chosen to include evaluation by averages of records, by averages of deviations from various contemporary averages [the expected values of some of these are discussed by VanVleck *et al.* (6)], and by percentage of daughter records exceeding various contemporary averages.

These methods are as follows for both milk and fat production:

I. Quantitative procedures

A. First records

(1) mean of first records of daughters,

(2) mean of deviations of first records from herd season averages,

(3) mean of deviations of first records from stable-mate averages,

(4) mean of deviations of first records from adjusted stable-mate averages.

B. Average records
   (5) mean of daughter averages,
   (6) mean of deviations of daughter averages from herd season averages,
   (7) mean of deviations of daughter averages from stable-mate averages,
   (8) mean of deviations of daughter averages from adjusted stable-mate averages.

II. Plus and minus procedures

A. First records
   (9) percentage of first records of daughters exceeding breed season averages,
   (10) percentage of first records exceeding herd season averages,
   (11) percentage of first records exceeding stable-mate averages,
   (12) percentage of first records exceeding adjusted stable-mate averages.

B. Average records
   (13) percentage of daughter averages exceeding season averages,
   (14) percentage of daughter averages exceeding herd season averages,
   (15) percentage of daughter averages exceeding stable-mate averages,
   (16) percentage of daughter averages exceeding adjusted stable-mate averages.

III. Adjusted quantitative procedures

   (17) mean of daughter averages adjusted for the regression of daughter records on adjusted stable-mate averages which is $[\overline{d} - b(\overline{X} - \mu)]$ as given by Equation (1) before adjustment for number of daughters, and
   (18) the control procedure.

ANALYSIS AND RESULTS

Product moment correlations were computed between the values obtained for each procedure and the control procedure for both milk and fat. The correlations associated with milk are shown in Table 1 and those concerning fat in Table 2 for all sires, regardless of number of daughters and for sires having at least 20, 50, and 200 daughters with first records. Approximate confidence limits for these correlations were computed by use of the z transformation, as given by Fisher (1), where $z = \log_e \left(\frac{1+r}{1-r}\right)^{\frac{1}{2}}$. The 95% confidence interval for z is $z \pm 1.96\sigma_z$, where $\sigma^2_z = \frac{1}{n-3}$ and n = number of observed pairs. Approximate confidence limits for r were then obtained by transforming the upper and lower limits of z back to r values. These limits are also listed in Tables 1 and 2. Inspection of Tables 1 and 2 reveals that the correlations for fat and milk are very similar. Therefore, discussion will be restricted to the evaluation procedures for milk production.

TABLE 1

Correlations between Evaluation Procedures 1, . . . , 17 and Procedure 18 for milk production and their 95% confidence levels

No. of sires	198			107			63			26		
N[a]	N > 0			N > 20			N > 50			N > 200		
Correlation coefficient	r_L[b]	r	r_U[c]	r_L	r	r_U	r_L	r	r_U	r_L	r	r_U
Quantitative procedures—first records												
$r_{1,18}$	.51	.60	.69	.77	.84	.89	.80	.87	.92	.86	.93	.97
$r_{2,18}$	.60	.68	.75	.89	.93	.95	.90	.94	.96	.86	.94	.97
$r_{3,18}$	.60	.69	.75	.89	.92	.95	.90	.94	.96	.86	.94	.97
$r_{4,18}$	.62	.70	.77	.90	.93	.95	.91	.94	.97	.86	.94	.97
Quantitative procedures—average records												
$r_{5,18}$	.59	.67	.74	.86	.90	.93	.89	.93	.96	.93	.97	.99
$r_{6,18}$	.70	.76	.81	.98	.98	.99	.99	.99	1.00	1.00	1.00	1.00
$r_{7,18}$	.70	.77	.82	.97	.99	.99	.99	.99	1.00	1.00	1.00	1.00
$r_{8,18}$	.72	.78	.83	.99	.99	.99	.99	1.00	1.00	1.00	1.00	1.00
Plus and minus procedures—first records												
$r_{9,18}$	.30	.42	.53	.77	.84	.89	.80	.88	.92	.81	.91	.96
$r_{10,18}$	.35	.47	.57	.79	.85	.90	.79	.87	.92	.80	.91	.96
$r_{11,18}$	.35	.47	.57	.79	.85	.90	.79	.87	.92	.80	.91	.96
$r_{12,18}$	.34	.46	.56	.80	.86	.90	.80	.87	.92	.79	.90	.96
Plus and minus procedures—average records												
$r_{13,18}$	.51	.60	.69	.84	.89	.92	.90	.94	.96	.94	.97	.99
$r_{14,18}$	.60	.69	.75	.92	.95	.96	.95	.97	.98	.98	.99	1.00
$r_{15,18}$	.60	.69	.75	.92	.95	.96	.95	.97	.98	.98	.99	1.00
$r_{16,18}$	.62	.70	.76	.93	.95	.97	.96	.97	.98	.98	.99	1.00
Adjusted quantitative procedure—average records												
$r_{17,18}$	.73	.79	.84	.99	.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[a] N = Number of daughters with first records per sire group.
[b] r_L = The lower bound of the 95% confidence interval of the correlation coefficient.
[c] r_U = The upper bound of the 95% confidence interval of the correlation coefficient.

DISCUSSION

The first eight procedures are based on quantitative measures of breeding value—four of these on first records only and four on means of daughter averages. The 17th method is also a quantitative measure. Part-whole relationships exist between these and the control procedure. The correlations with the control procedure increase with increasing similarity between the procedures. For example, the quantitative procedures expressed as deviations from herd, stable-mate, and adjusted stable-mate averages are correlated nearly the same with the control procedure. These deviations usually would be expected to be approximately the same. Even within this group of deviations the correlations with the control increase slightly as the procedures become more similar to the control. Deviations from adjusted stable-mate averages are more like the control than deviations from stable-mate averages which, in turn, are less dissimilar to the control than deviations from herd averages. Among the quantitative procedures the daughter average is least similar and Procedure 17 is most

TABLE 2

Correlations between Evaluation Procedures 1, . . . , 17 and Procedure 18 for fat production and their 95% confidence limits

No. of sires	198			107			63			26		
N[a]	N > 0			N > 20			N > 50			N > 200		
Correlation coefficient	r_L[b]	r	r_U[c]	r_L	r	r_U	r_L	r	r_U	r_L	r	r_U
Quantitative procedures—first records												
$r_{1,18}$	.52	.62	.70	.75	.82	.87	.76	.85	.91	.89	.95	.98
$r_{2,18}$	.56	.65	.72	.88	.92	.94	.87	.92	.95	.86	.94	.97
$r_{3,18}$	.57	.66	.73	.88	.92	.94	.87	.92	.95	.87	.94	.97
$r_{4,18}$	.58	.67	.74	.89	.93	.95	.89	.93	.96	.87	.94	.97
Quantitative procedures—average records												
$r_{5,18}$	.60	.68	.75	.82	.88	.91	.85	.91	.94	.93	.97	.99
$r_{6,18}$	.70	.77	.82	.97	.98	.99	.98	.99	.99	.99	1.00	1.00
$r_{7,18}$	.71	.77	.82	.97	.98	.99	.98	.99	.99	.99	1.00	1.00
$r_{8,18}$	.73	.79	.84	.98	.99	.99	.99	1.00	1.00	1.00	1.00	1.00
Plus and minus procedures—first records												
$r_{9,18}$	.21	.34	.46	.73	.81	.86	.77	.86	.91	.85	.93	.97
$r_{10,18}$	.34	.46	.56	.79	.85	.90	.79	.87	.92	.87	.94	.97
$r_{11,18}$	.34	.46	.56	.79	.85	.90	.79	.87	.92	.86	.94	.97
$r_{12,18}$	.34	.46	.56	.79	.86	.90	.78	.86	.91	.86	.93	.97
Plus and minus procedures—average records												
$r_{13,18}$	.53	.62	.70	.82	.88	.91	.85	.90	.94	.95	.98	.99
$r_{14,18}$	.61	.69	.76	.92	.95	.96	.93	.96	.98	.98	.99	1.00
$r_{15,18}$	.61	.69	.76	.92	.95	.96	.93	.96	.97	.98	.99	1.00
$r_{16,18}$	.63	.71	.77	.93	.95	.97	.95	.97	.98	.98	.99	1.00
Adjusted quantitative procedure—average records												
$r_{17,18}$	.74	.79	.84	.99	.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[a] N = Number of daughters with first records per sire group.
[b] r_L = The lower bound of the 95% confidence interval of the correlation coefficient.
[c] r_U = The upper bound of the 95% confidence interval of the correlation coefficient.

similar to the control procedure. The correlations also increase when means of daughter averages are used rather than first records. The control procedure is based on average records rather than first records.

Another factor to consider in comparing these procedures is the character being evaluated. Procedures 1-8 and 17-18 are based on prediction of a sire's breeding value for pounds of milk or fat. Methods 9-16 are not quantitative in the same sense. They are merely evaluations according to estimated probabilities that daughter records will exceed those of some group of contemporaries. The magnitude by which these records exceed or fall short of their contemporaries is not measured. In theory, it would seem that both types of procedures would give the same results. If, however, some sires produce a few very good or a few very poor offspring, then these extreme deviate records will tend to lower or raise the evaluation when the evaluation procedure is based on pounds of production, even though the very poor records or very good records are caused by extremes of environment. The plus or minus system minimizes the

effect of such extreme deviations. Yet, if the extreme deviations are truly genetic effects, then the plus or minus procedure will not measure the differences as accurately as the quantitative procedures. The plus or minus procedures also are not affected as much by faulty observations or errors of recording as are the quantitative procedures. Of course, fairly large numbers of records must be available or the plus and minus system also will be somewhat affected. An advantage of the nonparametric procedures is the computational ease of some of them. For example, the number above season average would be easy to calculate as would the number above herd averages. To be useful, however, such methods must be sufficiently accurate. It also should be noted that these procedures would be affected by faulty age-correction factors and by selection, as would the quantitative methods.

The correlations found in this study indicate that both types of procedures give essentially the same result but that more daughters are needed for the plus or minus systems to be as accurate as the quantitative procedures. Examination of Tables 1 and 2 reveals that the correlations between Procedures 1-17 and Procedure 18 are so low for the case where all sires are evaluated regardless of number of daughters as to preclude any consideration of these procedures for use in sire evaluation, if it can be safely assumed that the control procedure is the best. It should be noted that if the control procedure measures breeding value relatively inaccurately, then this conclusion would not be valid. The poorest relationships seem to exist for the plus and minus systems for first records with the control procedure.

If N is larger than 200, all procedures utilizing means of average records of daughters provide essentially the same evaluation as the control procedure. The methods which use first records alone are not correlated with the control procedure as much as expected. It appears, even with still larger N, that evaluation on first records will not give the same results as the control method, which suggests that some factor such as age-conversion factors or selection may be causing the difference.

CONCLUSIONS

If it can be assumed the procedure used for evaluating sires at the New York Dairy Records Processing Laboratory is the best, and if no restrictions are placed on the number of first records of a sire's progeny required for consideration, then none of the alternative procedures examined is sufficiently correlated with the control procedure for general use in sire evaluation. If, however, at least 50 first records are available from each sire group, the procedures utilizing averages of daughter averages provide nearly the same evaluation as the standard procedure. The computationally easy plus and minus systems may have merit if computational costs are high and if at least 50 daughter records are available per sire group.

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EFFECTS OF −196 and −76° C. STORAGE ON THE RESISTANCE OF BULL SPERM TO THREE REPEATED FREEZE-THAW TREATMENTS [1]

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SUMMARY

To determine the effects of three repeated freeze-thaw treatments on spermatozoon survival at −196 and −76°C. immediately after the initial freezing and after a 6-mo. storage period, a total of 30 ejaculates, three from each of ten Holstein bulls, were employed. The number of progressively motile sperm per milliliter of extender during the immediate and 6-mo. freeze-thaw treatments (−196 and −76°C.) were significantly different ($P < 0.01$) among bulls, between refrigerants, and between storage periods. After a 6-mo. period, spermatozoa stored in liquid nitrogen exhibited greater resistance to repeated freeze-thaw treatments than paired samples in dry ice–alcohol. This resistance difference was not observed during the immediate freeze-thaw treatments. The percentages of sperm lost between repeated freeze-thaw treatments were significant within each temperature. Correlations of sperm survival, after each freeze-thaw treatment at −196 and −76°C., with fertility of semen stored at −76°C., were not statistically significant, range + 0.17 to + 0.48 on a bull basis.

Investigators (6, 9, 14) have reported considerable variation in the resistance of different semen samples to withstand the stress of freezing and thawing. Sherman (13) demonstrated, using a tissue disintegrator, that there was no correlation between the physical strength of bull spermatozoa and their susceptibility to the lethal factor or factors of freezing and thawing. In a later study (14), it was reported that the survival of bovine spermatozoa exposed to repeated freeze-thaw cycles at a freezing temperature of −78° C. was not controlled by a simple probability relationship. This was in agreement with earlier findings involving influenza virus (7) and yeast cells (5).

Several attempts (1, 2, 4, 9) have been made to use various cold-shock treatments as a means of distinguishing superior semen samples. Bishop and Hancock (1) found that the more active bull spermatozoa were most resistant to cold shock and that samples with a high incidence of dead sperm were least resistant to cold shock. Docke (3) observed that sperm survival after a 10-min. cold shock in dry ice gave a reliable indication of the suitability of semen for low-temperature storage. Some workers utilizing cold-shock treatments have reported significant correlations between sperm motility and fertility (4, 9); however, others have found no significant correlations (2).

To date, no work has been reported on the susceptibility of bull spermatozoa to repeated freeze-thaw treatments at −196° C. Therefore, this experiment was

Received for publication October 16, 1960.

[1] Supported in part by grants-in-aid from Chas. H. Hood Dairy Foundation, and New England Artificial Breeding Council. Portions of this paper were presented at the North Atlantic Section meeting of the American Society of Animal Production, Amherst, Massachusetts, August 23-24, 1960.

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undertaken to compare the resistance of spermatozoa to repeated freeze-thaw treatments, at −196 and −76° C., immediately after freezing and after 6-mo. storage at their respective temperatures. An attempt was also made to correlate sperm survival after each freeze-thaw treatment, at each temperature, with fertility of semen stored at −76° C.

EXPERIMENTAL PROCEDURE

Three ejaculates from each of ten Holstein bulls routinely used in the New England Selective Breeding Association were collected with an artificial vagina. Immediately after collection of the semen, a per cent progressive motility estimation and hemocytometer count was made. The semen was extended in egg yolk, sodium citrate, and glycerol to contain a final concentration of 30 million progressively motile sperm per milliliter as follows: (a) extended to one-half the final volume, in a nonglycerol fraction at 32° C.; (b) allowed to cool to 5° C. over a 3- to 3.5-hr. period; and (c) glycerol-containing fraction of the extender was then added in four equal parts at 15-min. intervals. Final concentration of the egg yolk, sodium citrate, and glycerol extender was 20, 2.1, and 7%, respectively, which included antibiotics at the rate of 1,000 units of penicillin, potassium G, and 1,000 γ of dihydrostreptomycin sulfate per milliliter of semen. Toward the end of a 15-hr. equilibration period, the semen was hermetically sealed in 1.2-ml. glass ampules. Following the equilibration period, the ampules were placed on racks routinely used in liquid nitrogen storage (16), immersed in an alcohol bath at 5° C., and frozen at the rate (bath temperature) of 1° C. per minute from 5 to −15° C. and at 4° C. per minute from −15 to −76° C. (10). The ampules from each ejaculate were then appropriately divided for storage in liquid nitrogen and dry ice–alcohol. The ampules stored in liquid nitrogen were transferred, on racks, directly from the freezing bath at −76° C. to liquid nitrogen refrigerators (Linde Model LNR-25B) maintained at −196° C.

Following a 2.5-hr. adjustment period, 12 ampules, six per ejaculate from each storage temperature, were thawed in ice water. Two ampules from each storage condition were removed from the ice water and per cent progressive motility estimations made in duplicate. This constituted the first (I) freeze-thaw treatment. The remaining eight ampules, four per storage treatment, were refrozen after a 20-min. thawing period, at the routine rate, and transferred to their original refrigerant. Following a similar adjustment period, the refrozen samples were again thawed in ice water and motility estimations recorded from two ampules at each storage temperature. This constituted the second (II) freeze-thaw treatment. After a 20-min. thawing process, the remaining four ampules were refrozen as previously outlined and transferred to their respective temperatures. The samples were permitted to adjust for 30 min. to storage temperature and then thawed and progressive motility estimated. This completed the third (III) freeze-thaw treatment. A similar procedure was repeated after a 6-mo. continuous storage period at their respective refrigerant temperatures.

For statistical analysis, the progressive motility estimations were converted to the number of progressively motile sperm per milliliter of extender by multiplying the total number of sperm per milliliter of extender by the per cent progressive motility and dividing by 100.

Fertility data (60-90 day per cent nonreturn) were obtained by requesting 37 technicians to breed 30 unselected first service cows, one to semen from each ejaculate stored continuously in dry ice–alcohol. This resulted in approximately 33 first services per ejaculate for correlation determinations between fertility and the number of progressively motile sperm surviving each freeze-thaw treatment.

RESULTS AND DISCUSSION

The number of progressively motile sperm per milliliter of extender following each of three repeated freeze-thaw treatments at −196 and −76° C. immediately after the initial freezing and after a 6-mo. storage period are presented in Table 1.

TABLE 1

The number of progressively motile sperm surviving repeated freeze-thaw treatments immediately after initial freezing and after 6 mo. of storage
(Mean of three ejaculates per bull)

Bull	Dry ice Treatment			Liquid nitrogen Treatment		
	I	II	III	I	II	III
	(10^6 per milliliter of diluent)					
	After initial freezing					
A	12.50	5.42	2.67	11.75	5.75	2.17
B	23.42	17.42	10.83	23.42	18.92	10.50
C	19.50	15.58	9.75	20.33	16.33	9.75
D	14.08	3.83	1.25	14.08	4.17	1.50
E	17.17	11.92	4.92	17.50	12.25	5.67
F	6.75	2.25	0.33	7.92	1.58	0.50
G	16.58	9.92	3.75	16.58	12.00	4.50
H	18.00	9.33	3.67	18.75	10.08	4.83
I	19.25	16.75	10.67	18.92	16.00	11.00
J	21.67	13.50	10.67	21.67	13.75	10.67
Mean	16.89	10.59	5.85	17.09	11.08	6.11
S.D.[a] =	3.04	2.79	2.04	3.04	2.85	1.98
	After 6 mo. of storage					
A	1.67	0.58	0.17	6.83	1.17	0.50
B	10.08	3.50	1.25	17.42	11.50	3.17
C	9.83	3.00	0.92	15.33	10.75	3.50
D	2.75	0.42	0.17	6.83	1.42	0.25
E	6.67	1.92	0.42	15.33	9.83	2.25
F	1.50	0.33	0.08	6.00	0.50	0.25
G	4.25	1.33	0.25	11.92	6.67	1.83
H	6.42	2.17	0.42	11.08	5.33	1.00
I	12.67	4.92	1.42	17.17	12.83	6.33
J	8.83	3.00	0.67	17.75	10.33	4.50
Mean	6.47	2.12	0.58	12.57	7.03	2.36
S.D.[a] =	2.61	1.35	0.29	2.09	2.07	1.35

[a] S.D. = Standard deviation of an ejaculate calculated from the among ejaculates within bulls variance.

An analysis of variance of these data indicated a significant difference ($P < 0.01$) among bulls, between refrigerants, and between storage periods. Similar differences have been previously reported on motility studies with frozen semen, not involving the additional stress of freeze-thaw treatments (8, 11). One factor contributing to the agreement of these studies employing different procedures lies undoubtedly in the fact that a high positive association existed between the effects of the initial freeze-thaw treatment and subsequent treatments.

In all cases, little difference in sperm resistance was observed between the two refrigerants during the immediate freeze-thaw treatments; however, after a 6-mo. storage period, this difference was of a much greater magnitude. This is attributed to the greater resistance exhibited by spermatozoa to repeated freeze-thaw treatments after a 6-mo. storage in liquid nitrogen than was exhibited by semen from split-ejaculates in dry ice–alcohol. These observations, plus those of previous studies (8, 11), indicate that sperm injury as measured by immobilization resulting from freezing, storing, and thawing at −76° C. is of greater consequence than at −196° C.

TABLE 2

First services and per cent 60-90 day nonreturn for semen stored in dry ice–alcohol

Bull	No. of ejaculates	First services	Per cent 60-90 day nonreturns
A	3	89	61.8
B	3	96	62.5
C	3	95	65.3
D	3	96	54.2
E	3	103	55.3
F	3	93	55.9
G	3	106	58.5
H	3	97	56.7
I	3	103	57.3
J	3	95	55.8
Total	30	973	Av. 58.3

Standard deviation among ejaculates within bulls = 7.51.

The mean percentage of sperm lost during the three immediate freeze-thaw treatments were 41.9, 39.3, and 49.4 vs. 42.4, 40.5, and 49.9 at −196 and −76° C., respectively. Statistical analysis of these percentages showed a significant difference ($P < 0.05$) between treatments.

These results are consistent with the observations made by Sherman (14) and support his conclusion that sperm survival subjected to repeated freeze-thaw cycles is not controlled by a simple probability relationship. It was also suggested by Sherman that such factors as weakening of cells, deleterious effects other than immobilization, and the selection of resistant cells by survival may be involved in the survival of sperm subjected to repeated freeze-thaw treatments.

The fertility results by bull are presented in Table 2. Inspection of these data indicate that the conception rate of the field trial was inferior to that

reported in previous frozen semen studies (2, 4, 6, 8, 12). The authors feel this may be attributed to factors which include a temperature fluctuation during the equilibration period, the method of dispensing the semen to the technicians, and the small number of services made to each ejaculate.

TABLE 3

Correlations of the number of progressively motile sperm per milliliter of extender after repeated freeze-thaw treatments with fertility

Treatment	Refrigerant	Storage time	Correlation	
			Bull[a]	Ejaculate[b]
I	Dry ice	Post freeze	0.31	0.29
II	Dry ice	Post freeze	0.44	0.30
III	Dry ice	Post freeze	0.42	0.20
I	Dry ice	6 Mo.	0.28	0.25
II	Dry ice	6 Mo.	0.27	0.27
III	Dry ice	6 Mo.	0.40	0.11
I	Liquid nitrogen	Post freeze	0.31	0.29
II	Liquid nitrogen	Post freeze	0.48	0.27
III	Liquid nitrogen	Post freeze	0.36	0.18
I	Liquid nitrogen	6 Mo.	0.23	0.19
II	Liquid nitrogen	6 Mo.	0.28	0.23
III	Liquid nitrogen	6 Mo.	0.17	0.18

[a] Based on ten bulls, $r_{.05} = 0.63$.
[b] Based on 30 ejaculates, $r_{.05} = 0.36$.

Correlations between the number of progressively motile sperm per milliliter of extender after each freeze-thaw treatment and fertility of frozen semen stored in dry ice–alcohol are summarized in Table 3. All correlations by bull or by ejaculate were not significant ($P = 0.05$); however, all were positive and of relatively equal magnitude. It may also be seen from Table 3 that the correlations by bull between fertility and the number of progressively motile sperm per milliliter of extender were generally higher than the corresponding ejaculate correlations. This was expected, because much of the ejaculate variability was eliminated by using bull averages. These observations suggest that an association may exist between fertility and the treatment imposed in these studies, but that the numbers employed were not of sufficient size to establish it statistically.

The observations that all correlations between fertility and the number of progressively motile sperm were positive suggest that, following some modifications in procedures, the use of repeated freeze-thaw treatments may be beneficial to artificial breeding organizations in selecting high quality frozen semen samples.

ACKNOWLEDGMENTS

Gratitude is extended to the personnel of the New England Selective Breeding Association and especially to Mr. Paul Heller, Manager, for the use of their facilities.

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ERRATUM

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Table 1. Error, d.f. should be 2354.

TECHNICAL NOTES

PARTIAL PURIFICATION OF THE B-ESTERASE OF BOVINE MILK

Tenfold purification of the B-esterase (1) of bovine milk has been obtained by adsorption of the enzyme on a $Mg(OH)_2$ suspension, followed by decomposition of the $Mg(OH)_2$ to a soluble carbonate with carbon dioxide under pressure and removal of ions by dialysis.

Details of the purification procedure were as follows: Milk known to be high in B-esterase activity was selected. It was withdrawn from the glass jar at the milking parlor into a brown glass bottle, immediately placed in ice water, brought to the laboratory, and gassed with nitrogen (2). It was stored in an atmosphere of nitrogen, at 2-5° C., until the following day. All subsequent operations, except assays for enzyme activity and nitrogen, were conducted in the cold room (2-5° C.). One hundred and sixty milliliters of gravity skim, obtained from the above milk, were added to 40 ml. of $Mg(OH)_2$ suspension (3) in a 250-ml. centrifuge bottle and shaken vigorously for 30 sec. The mixture was then centrifuged (800 g) for 10 min., the supernatant discarded, and the sides of the centrifuge bottle rinsed with water while the bottle was inverted. Forty milliliters of water was added to the bottle, the $Mg(OH)_2$ pellet was dispersed by shaking and again centrifuged down (15 min. at 800 g). Again, the supernatant was discarded. The $Mg(OH)_2$ was immediately transferred to a stainless steel pressure cylinder by rinsing the centrifuge bottle with three successive 30-ml. portions of water. After flushing with carbon dioxide, a pressure of 30 p.s.i. of this gas was applied for 30 min., during which time the cylinder was continuously shaken in a horizontal plane. At this point, if the $Mg(OH)_2$ was completely decomposed, the preparation foamed considerably when the pressure was released. A trace of foam depressant was added, the liquid transferred to cellophane sacs, and dialyzed against repeated changes of water for 48 hr. During the above steps, an effort was made to keep the time of contact between enzyme and $Mg(OH)_2$ to a minimum.

Degree of purification was calculated on the basis of esterase activity per milligram of nitrogen. Esterase activity was estimated using Warburg manometric techniques (1), at pH 8.0 and 37° C., with tributyrin as substrate. The reaction mixture contained eserine sulfate (10^{-5} *M*) to inhibit any cholinesterase present. One milliliter of heated milk (75 ±5° C. for 10 min.) was added to reaction mixtures when the purified preparation was being assayed. Nitrogen content of the skimmilk and esterase preparations were determined by a semimicro-Kjeldahl method.

Five-to tenfold purification of the B-esterase of milk has been obtained repeatedly, using the methods described above. Two recent preparations showed an average purification of 10.7-fold and a yield of 44% of the original activity. Several preparations have been lyophilized with only slight loss in activity. One of these shows no activity loss after storage at 2-5° C. for 2 mo.

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SIMPLE METHOD FOR EXTRACTING FAT FROM WHOLE MILK POWDER

In connection with a study of off-flavor constituents in foam-dried whole milk powder, a simple, rapid method has been developed which quantitatively extracts the fat from both foam- and spray-dried powders. Previous work in this regard has been recorded by Lampitt and Bushill (2) and Whitney and Tracy (3). However, both of these methods were deemed unsuitable for our purpose, mainly because of the relatively long procedural times involved.

EXPERIMENTAL PROCEDURE

Fifty milliliters of methanol (Baker's[1] ACS grade) and 20 g. of powder were mixed with a glass rod until fluid enough (about 5 min.) to be stirred magnetically (15 min.). The methanol extract was filtered from the powder through S & S 507 paper using light suction. The dry powder was returned to the beaker and stirred with 100 ml. n-hexane (Phillips,[1] high grade) for 15 min. The slurry was filtered as above and the hexane extraction repeated once more. All solvents were removed on the steam bath, the residues taken up in about 75 ml. of methylene chloride (Fisher,[1] highest purity), dried over anhydrous sodium sulfate, and filtered through sintered glass into tared vessels. After removal of the solvent, the fat was dried to constant weight in a vacuum desiccator. For routine analyses it is not, of course,

[1] The mention of trade names in this paper is for identification and implies no endorsement of the products.

TABLE 1

Recovery and distribution of fat from whole milk powders [a]

Whole milk powder	Type	Trial No.	Total fat recovered [a]	Per cent of total fat recovered in		
				Methanol	1st Hexane extraction	2nd Hexane extraction
			(%)			
Commercial a	Spray	1	99.58	9.67	88.02	2.31
		2	99.70	5.14	92.57	2.29
USDA	Foam (2 mo. old)	1	95.94	6.62	86.78	6.59
		2	96.12	6.69	85.92	7.38
USDA	Foam (2 yr. old)	1	96.72	7.30	83.29	9.40
		2	96.78	8.32	85.56	6.11
Commercial b	Spray	1	96.81	6.54	91.53	1.92
		2	93.88	5.75	89.74	4.50
USDA	Foam (fresh)	1	97.52	13.37	85.59	1.04
		2	97.71	21.03	76.36	2.61

[a] Recoveries based on the average of closely agreeing duplicates obtained by the Mojonnier method.

necessary to weigh the individual extracts. It is done here solely for the purpose of showing the fat distribution in each extract. In practice, the extracts may be combined and evaporated.

RESULTS AND DISCUSSION

Results of the study are presented in Table 1. The wide variation in the amount of fat obtained in any one extraction probably is due to differences in the amount of vacuum applied, more fat being carried into the methanol filtrate mechanically with higher vacuum. The end result, however, is the same as manifested by the good agreement between duplicates.

Scaling the procedure up (100 g. powder) resulted in a yield of 99% on fresh foam-dried powder. No recovery studies were made on less than 20 g. powder. The procedure appears flexible enough to permit the use of solvents other than n-hexane, although we did not investigate this point. Substituting n-propanol for methanol gave very poor yields. The use of hexane purified by the method of Bassette (1) furnished fat with no detectable solvent odor, which ostensibly can be used for organoleptic evaluation.

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DICHLOROFLUORESCEIN AND POTASSIUM CHROMATE AS INDICATORS IN A TITRATION TEST FOR SALT IN CHEESE [1]

The common laboratory procedure used for measuring salt in cheese is the modified Volhard method, described in 1937 by the Committee on Cheese Analysis of the American Dairy Science Association (A.D.S.A.) (6).

A direct titration method for measuring the salt content of butter was proposed by Sammis (3) for factory practice. Marquardt (2) adapted the Sammis technique to the analysis for salt in a water dispersion of cheese and compared this test for salt in cheese with the modified Volhard method. It was found that the extent of proteolysis of cheese adversely affected the direct titration, because water-soluble and suspended protein in concentrations above 0.2 g. per 100 ml. of solution introduced an error proportional to the amount of protein in the aliquot tested. Consequently, it was recommended that the test be used only for cheese less than five days old. Potassium chromate and dichlorofluorescein (DCF) produced comparable results when used as end point indicators in this direct titration method.

Skelton and Bryant (4) and Weckel (5) found that dichlorofluorescein gave a sharper end point and greater accuracy than potassium chromate in the direct titration test for salt in butter.

[1] Approved for publication by the Director of the Agricultural Experiment Station, University of Wisconsin.

This study was undertaken to compare the potassium chromate and dichlorofluorescein indicators in a direct argentometric titration of a water dispersion of Cheddar Cheese, and to observe the effects of ripening on the results of the titration test. The A.D.S.A. method was used as a reference test (6).

METHODS

Sampling. Cheese to be analyzed was sampled in accordance with A.O.A.C. methods (1). The cheese was ground and mixed in an Osterizer blender. A few samples which formed a gummy mass in the blender jar were pressed through an eight-mesh wire screen and mixed thoroughly by hand in the sample jar.

Direct titration with chromate indicator. Figure 1 shows the apparatus used. A 10-g. sample was weighed in an evaporating dish and transferred to a one-half-pint Mason jar with approximately 50 ml. of warm (50-60° C.) distilled water. The jar and sample were placed on the blender at low speed for 4 to 5 min., or until a homogeneous suspension was obtained. The suspension was transferred quantitatively to a 250-ml. graduated cylinder, diluted to 250 ml. with distilled water, mixed thoroughly, and allowed to stand for 5 to 10 min. while the suspended cheese settled out. To a 25-ml. aliquot of the supernatant was added 2 ml. of a 2% solution of potassium chromate in distilled water. Then the mixture was titrated with a 0.01711 *N* solution of silver nitrate made by dissolving 2.906 g. of $AgNO_3$ in distilled water to make one liter of solution. The first discernible color change due to the red color of precipitated silver chromate was taken to be the end point. Using this procedure, 1 ml. of this silver nitrate solution is equivalent to 0.1% salt.

FIG. 1. Apparatus for direct titrations showing (from left): triple-beam balance (sensitivity, 0.01 g.) with dish for weighing the sample; blender; 250-ml. graduated cylinder; burette stand with 25-ml. pipette, 10-ml. burette (0.05-ml. intervals), and magnetic stirrer; and bottles of dichlorofluorescein and potassium chromate indicator solutions.

Direct titration with dichlorofluorescein indicator. The procedure was the same as that used with the chromate indicator, except that five drops of a 0.1% solution of dichlorofluorescein in 70% ethanol was added as the end point indicator and the sample was titrated to the first definite shade of pink.

Experimental procedure. The methods of analysis were first compared by testing 5-lb. midget Cheddars which had been made for this purpose. These small Cheddars were divided into four groups, each of which was stored at a different temperature for 7 wk. Temperatures of storage were −15, 38, 46, and 55° F. Cheeses were removed from storage and analyzed for salt at each of ten intervals beginning at one day and ending at 49 days of aging. Low temperatures and short holding times minimized the aging effect, whereas higher temperatures and longer holding induced more extensive proteolysis.

RESULTS

Duplicate analyses were run on all samples by the direct titration test with chromate as indicator, and by the A.D.S.A. method, and beginning at 15 days of age all samples of cheese were also analyzed by the direct titration method, using dichlorofluorescein as indicator.

The mean percentages of salt measured by direct titration with chromate indicator were 1.66, 1.62, 1.68, and 1.71% for cheese stored at −15, 38, 46, and 55°F.; comparable tests by the A.D.S.A. method averaged 1.54, 1.49, 1.51, and 1.51%.

Table 1 shows the differences between the tests made with the chromate indicator and those made by the A.D.S.A. method at each interval of aging and at each temperature of aging. The test using chromate as indicator always gave higher percentages of salt than the A.D.S.A. test.

These differences were significant ($P = 0.01$) at each of the four curing temperatures. The means of these differences were 0.12, 0.13, 0.17, and 0.20% at temperatures of −15, 38, 46, and 55° F., respectively. Differences between the means of differences associated with storage at 38 and 55° F. were significant ($P = 0.05$), but all other means were not significantly different.

No definite relation could be detected between the differences in methods of testing and the duration of cheese storage; differences were essentially the same on the fresh and held lots of cheese.

Table 2 shows that the direct titration test

TABLE 1

Differences[a] in NaCl measured by direct titration with chromate indicator and by A.D.S.A. procedures (direct titration minus A.D.S.A.)

Age of cheese	Storage temperature			
	−15° F.	38° F.	46° F.	55° F.
(days)	*(%)*			
1	0.18	0.24	0.18	0.20
3		0.16	0.26	0.21
6	0.11	0.17	0.19	0.14
8		0.09	0.13	0.16
10		0.09	0.13	0.10
15	0.07	0.09	0.15	0.15
22		0.11	0.10	0.22
29		0.08	0.10	0.23
36	0.10	0.09	0.19	0.30
49		0.15	0.25	0.28
Mean of differences	0.12	0.13	0.17	0.20
Standard error	0.023	0.016	0.018	0.021
t	4.94[b]	8.06[b]	9.39[b]	9.66[b]

[a] Each difference was calculated from the means of duplicate tests by each method.
[b] Significant, P = 0.01.

TABLE 2

Differences[a] in NaCl measured by the direct titration with dichlorofluorescein indicator and by A.D.S.A. procedures (direct titration minus A.D.S.A.)

Age of cheese	Storage temperature			
	−15° F.	38° F.	46° F.	55° F.
(days)	*(%)*			
15	0.01	0.01	0.02	0.02
22		−0.04	0.02	0.04
29		0.00	0.01	0.02
36	0.00	0.00	0.01	0.02
49		0.01	0.00	−0.01
Mean of differences	0.005	−0.004	0.012	0.018
Standard error		0.010	0.004	0.012
t		−0.40[c]	3.21[b]	1.51[c]

[a] Each difference was calculated from the means of duplicate tests by each method.
[b] Significant, P = 0.05.
[c] Not significant.

with dichlorofluorescein as indicator gave excellent agreement with results by the A.D.S.A. method on cheese of all ages and at all temperatures of holding. Means of differences between tests ranged from −0.004% on cheese stored at 38° F. to 0.018% on cheese stored at 55° F.

All three test procedures were next used to analyze 43 samples of cheese from commercial sources; it was expected that such samples would have a broader range of salt concentration as well as greater differences in age, quality, and composition. The lots of cheese were selected at random from 23 Wisconsin factories; they included 40 samples of Cheddar, two of Colby, and one of processed Cheddar Cheese. Fifteen of the Cheddar samples were approximately 1 mo. old or less, 22 were 4 to 6 mo. old, and 3 were over 1 yr. old, with a maximum of 17 mo. The two Colby samples were less than 1 mo. old. Percentages of salt measured by the A.D.S.A. method ranged from 1.41 to 2.71%.

Means and standard deviations of the percentages of salt in the 43 samples as measured by each method were 1.74 ± 0.25 by the A.D.S.A. method, as compared with 1.73 ± 0.24 by the DCF test and 1.92 ± 0.25 by the chromate test. The results of the titration test with the chromate indicator differed significantly (P = .01) from those obtained by the two other methods; the latter were identical.

Means and standard deviations of differences between the duplicate tests by each of three methods were calculated to evaluate the uniformity of each procedure. The differences between duplicates averaged 0.018% ± 0.012 by the A.D.S.A. method, 0.022% ± 0.016 when dichlorofluorescein was used as indicator, and 0.025% ± 0.021 with the chromate indicator. Listed in the same order, the maximum differences observed between duplicates by the three methods were 0.06, 0.08, and 0.11%.

The agreement between the results of A.D.S.A. test (Y) and the titration test with DCF as indicator (X) was excellent: the coefficient of correlation was 0.97, the regression equation $Y = -0.029 + 1.017X$, and the standard error of estimate 0.06. The agreement between the A.D.S.A. test (Y) and the titration test with chromate as indicator (X) was not close enough to justify recommending the use of the chromate indicator for the direct titration test; the coefficient of correlation was 0.88, the regression equation $Y = 0.058 + 0.875X$, and the standard error of estimate 0.10.

Figure 2 shows the distribution of samples according to the unsigned differences between the A.D.S.A. test and each of the direct titration tests made on the 43 commercial samples. A majority (27) of the samples tested by the DCF method fell in the lowest class interval of differences of 0 to 0.04%; whereas, the largest number of samples tested by the chromate method fell in the class interval of differences of 0.10 to 0.14%. Differences between the A.D.S.A. and DCF methods did not exceed 0.16%, whereas the process cheese sample tested by the chromate method differed by 0.75% from the sample tested by the A.D.S.A. method.

The chromate end point lacks clear definition between the yellow color of the solution being titrated and the red color of the end point, so that errors in reading amounting to 0.1% salt are quite possible. The dichlorofluorescein indicator gives a sharp end point to the titration.

The 10-g. sample used in the direct titration

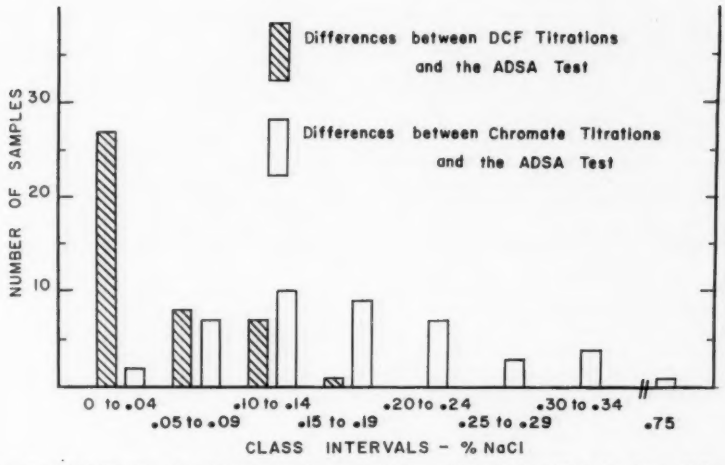

FIG. 2. Distribution of cheese samples tested for salt and classified to show differences, without regard to sign, between the direct titration tests and the A.D.S.A. tests made on identical samples. A total of 43 samples was tested by each method.

test can be weighed quickly and with sufficient accuracy on the triple-beam scales or torsion balance commonly found in dairy plant laboratories. This is an important factor in testing where equipment availability and costs are important.

The time required for analyzing a large number of samples by the A.D.S.A. method is shorter when sufficient hot plate surface is available for digestion of samples. Time for testing for salt by the A.D.S.A. method also is saved when the same samples of cheese are first tested for moisture; the moisture test residue can be used for the A.D.S.A. test so that one weighing suffices for both tests. The direct titration test is shorter when a small number of tests must be made.

This study indicates that the direct titration salt test for Cheddar, using dichlorofluorescein as indicator, is sufficiently accurate for composition and quality control in factory and warehouse practice.

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ACKNOWLEDGMENT

Thanks to L. D. Schreiber & Company, Green Bay, Wisconsin, for providing the cheese and the facilities for sampling.

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PREFERENCE OF CALVES FOR A CONCENTRATE FEED WITH AND WITHOUT ARTIFICIAL FLAVORS[1]

Stimulating the tendency for young calves to consume solid feeds could promote a quick transition from fluid milk or milk replacer to solid feeds. Yet, few reports on the effects of flavor in the feed of calves are available. Norton and Eaton (3) observed a measurable increase in palatability of a calf starter upon increasing the molasses content from 5 to 12.5%. A significant decrease in acceptability due to anise oils occurred when Miller *et al.*

[1] Florida Agricultural Experiment Station Journal Series, No. 1126.

(2) offered simultaneously to calves a starter with and without this substance. Stubbs and Kare (4) reported that calves appeared to show a marked relish for a dilute sucrose solution. Later, Kare (1) reported that calves consumed a larger amount of sugar-coated than of plain feed for a period of 2 to 3 wk.

The present study employed ten newborn calves in the Florida Agricultural Experiment Station herd. The purpose was to determine whether young calves would select more of a flavored or plain feed when given a choice of the two.

All calves were removed from their dams at birth and confined to wooden pens which were 5 ft. wide and 10 ft. long. The rear half was protected on three sides by plastic-coated wire and from above by a sloping metal roof. Two double-compartment feed boxes were supplied. The feeders were constructed of plywood which was completely covered with fiberglass to prevent the absorption of flavors. One compartment of each feed box was kept filled with pangola grass hay and the other with plain or flavored feed. The feeders were changed at random daily to various parts of the pen to prevent possible bias of the data by calves habitually eating at the same location. The concentrate feed contained 300 parts by weight of ground snapped corn, 200 dried citrus pulp, 125 ground oats, 50 wheat bran, 100 cottonseed meal (41% protein), seven common salt, and 14 parts of steamed bone meal. In addition, the experimental feed contained 1 lb. per ton of a flavoring material which contained sodium saccharin and sodium cyclamate, ethyl lactate and other esters, vanillin and other aldehydes, citric acid and other organic acids, gamma nonyl lactone, diacetyl, vegetable gum, and sugar. Fresh water was provided in each pen at all times.

The milk feeding schedule included colostrum from birth through four days of age, a mixture of one-half colostrum and one-half reconstituted skimmilk, containing 1 lb. of air-dried solids per gallon, for five days through 21 days, and skimmilk alone through 60 days of age. Thereafter, only concentrates and hay were offered. All milk was fed at the rate of 9% of body weight in two equal portions daily. Daily feed consumption was recorded. For purposes of this report, the total concentrates consumed by each calf for 30-day periods is presented in Table 1.

Two calves appeared to prefer nonflavored feed. This preference was not particularly marked, and the average consumption of flavored feed was considerably greater than was consumption of the plain feed throughout the experiment.

Total consumption of flavored feed during the experiment was 1,448.2 lb., as compared to 1,220.9 lb. for the plain feed. Mean squares (with degrees of freedom) for treatment and treatment by animal interaction were 861(1) and 110(9), respectively. Differences were significant ($P < 0.025$). The preference for flavored feed was particularly marked during the period between 31 and 60 days; treatment by period mean square was significant ($P < 0.05$) when compared to treatment by period by animal mean square. Period and period by animal interactions were significant, ($P < 0.01$) and ($P < 0.05$), respectively. Their mean squares and the mean square for residual were 28,263 (2), 145(2), and 27(9).

This study indicated the preference of individual calves for the concentrate feed to which flavors were added. It did not imply that greater concentrate consumption would occur by calves fed the flavored concentrates com-

TABLE 1

Consumption of plain and flavored feed by calves to 90 days of age

Calf No.	Days on experiment					
	0-30		31-60		61-90	
	Plain	Flavored	Plain	Flavored	Plain	Flavored
	(lb.)					
1	4.6	4.1	19.1	28.1	45.8	74.2
2	14.8	17.7	18.3	26.8	46.6	66.4
3	13.6	17.5	27.3	36.7	65.9	98.6
4	23.2	16.3	29.6	25.5	41.1	37.6
5	9.8	11.9	14.3	13.9	23.3	17.8
6	7.3	10.0	24.9	28.1	60.3	62.7
7	1.4	2.5	19.0	21.9	49.8	62.4
8	17.8	21.0	47.5	51.9	113.7	121.7
9	30.1	40.5	36.3	64.7	127.3	163.2
10	36.4	42.9	64.8	72.8	187.0	188.8
Total	159.0	184.4	301.1	370.4	760.8	893.4

pared to calves offered the concentrates without flavoring additives.

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ACKNOWLEDGMENTS

The author wishes to thank Drs. J. K. Loosli and C. J. Wilcox for their assistance in the design and analysis of the experiment. Acknowledgment is made to Flavor Corporation of America, Chicago, Illinois, for the flavoring materials and for partial financial support of this work.

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OUR INDUSTRY TODAY

ADVERTISING AND MERCHANDISING RESEARCH IN THE DAIRY INDUSTRY [1]

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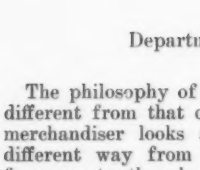
The philosophy of merchandising research is different from that of technical research. The merchandiser looks at business in a slightly different way from most other people, even from most other businessmen. They are not so much interested in producing a product as they are in producing a market.



Jack Weber

The merchandiser does not ask, "How can we sell the products we have?" so much as he first asks, "How can we produce a product that sells?" and then, "How is the best way of selling it?"

The merchandiser realizes that an industry can have the finest source of raw material . . . the most highly trained production force and the most efficient plant . . . the finest quality product that can be made . . . But . . . if the consumer doesn't want it, these other things are not long for this world.

Merchandisers are social scientists, they deal with people, their wants, desires, and reactions under certain conditions . . . while production people are physical scientists who deal with things rather than people. This is the most important consideration in realizing the difference in the approach to research that the merchandising-advertising-promotion people have, as compared to the technical research workers in any industry, including the dairy industry.

One way of expressing this would be to say that when a researcher deals with things he can be certain that within definable limits the truths that he works with are true today and tomorrow, and he can predict with relative certainty that given the same set of circumstances or factors, the outcome will be the same. This recognizes, of course, that our physical world around us is changing, it is evolving, but the process is so slow that it makes no practical difference in our work-a-day world.

The same is not true when you are dealing with people—their wants, their desires, their reactions to certain situations. What is true today may not very well be true tomorrow. Outside of the realm of philosophic discussion it is impossible to determine whether the fact that the same people react differently under apparently the same conditions at different times is due to an inherent quality called free will which enables them to react differently at their own desire, or whether this changing of reaction to the same stimulus is because the subject under consideration is never the same two moments in time, due to the fact that having lived knowledgeably the first moment, this gives him a different base on which to make decisions the second moment. In either case, the fact remains—the merchandising researcher is dealing with people, a dynamic, decision-making entity, rather than with static things. He must interpret his research in a different way from the physical scientist.

We can see the results of this inexplicable, dynamic changing of consumer decisions in a whirl around us. In the late '30's and early '40's the cigarette industry was used as a horrible example of a completely closed market into which no new brand of cigarettes could hope to enter and compete with the established brands. It was stated as a fact then, that as consumers got more money the predominance of the leading brands would grow greater and the low-price brands, such as Wings and Kools, would pass out of the market. It was ceded that price was the only thing that kept anyone from buying the popular cigarettes. But that situation changed over night. Not only have ten or 15 new brands become popular, but they are selling for more money, and they are selling in a form, with filters, that the seasoned old cigarette veterans said would never come about. It is obvious what caused the change . . . the cancer scare. The cigarette industry as a whole, however, didn't let the cancer scare get it down. The merchandisers brought out new brands so consumers could associate their regular old-line brand with cancer and still have a new noncancerous brand . . . they put filters on them, they put charcoal in the filters, they recessed the charcoal, and they supplied the public with what it wants—cigarettes, but cigarettes that had changed so as to be acceptable to the public in the context of the awareness of cancer. The industry as a whole has had

[1] The two papers in this section complete publication of the 11 papers presented to the Dairy Manufacturing Extension Section, at the 55th Annual Meeting of the American Dairy Science Association, Utah State University, Logan, 1960. These papers appeared in the December, 1960, January, February, March, and April, 1961, issues of this JOURNAL and will be available as reprints under one cover. The cost for 100, 200, 300, 400, and 500 reprints will be 116, 148, 181, 213, and 245 dollars, respectively. The print will be held for three months—E. O. HERREID.

the highest sales in its history since the cancer scare.

Another example is the American automobile. Compact cars are not a new idea. The American Austin, a small, cheap, European-type car was put out from 1932 to 1936 with poor acceptance. In '39 and '40 both Studebaker and Willys-Overland put out compacts that nearly broke them. And, yet, these were the times when a relatively inexpensive car should have appealed to people. In '45 there was the Crosley, in '50 the small American, and in '53 the small Willys and Henry J., none of which were accepted by consumers. But in 1956 American Motors tried it again with the Rambler, and sales started up. Small foreign cars came in in droves. But the American auto industry, sitting back and saying, "It's just a fad," lost half a million sales a year before it finally came out with its own compacts which now account for 26% of all sales and are estimated to reach 40% of sales in the next 2 yr. The American consumer's taste had changed in automobiles, but management hadn't kept up with this change until it was forced to.

These are two rather extreme cases in which whole industries have had to reorient thinking, production, and selling methods to the merchandising concept of "How can we produce a product that sells?"

The merchandising concept when applied to the dairy industry would not ask, "How can we sell butter?" but, "How can we make butter so that it will sell?"

It would not ask, "How do we make older people drink more milk?" but would ask, "How can we make milk so that people continue to drink it?"

It would not ask, "How do we sell more dairy products?" but would ask, "What new dairy products can we make that people will buy?"

After these questions were answered then the merchandiser would ask, "What is the easiest way to sell these products that the people want?"

The merchandiser would question the wisdom of an industry which brings political pressure to pass laws that will eventually bring on the death of that industry. Such laws as those that require high butterfat content in milk, which consumers do not want. Such laws as those that require a product that is labeled Cottage Cheese to have at least 12% butterfat in the dressing, when it has been repeatedly demonstrated that consumers do not want butterfat. Such laws as those that levy a high tax on a competing product when the product they put on the market is not acceptable to consumers in taste, in use characteristics, or in price.

The merchandiser and his research would start with the consumer and would build his products and sales methods to suit the consumer, not himself or his business operation. This difference in viewpoint, how one considers the consumer in the scheme of business operation, is the difference between technical and merchandising research.

We can divide merchandising-oriented research into two types; one deals with people and is usually carried on by a psychologist, social scientist, or economist; the other deals with products, and is carried on by technical dairy people.

Ideally, in the sequence of events of merchandising-oriented research, consumers are studied first to determine what new products, or new forms of old products, they will buy. In the second step, the technical people develop the new products or revamp the old ones to consumers' tastes. In the third step, various methods of selling are tried to determine which is most effective in getting consumers to buy what they want.

This, of course, is an oversimplification of the research process. Some steps are possible to skip, others can be handled from past experience; in still other cases two or three of the steps are carried on simultaneously.

Let's review some merchandising research that has been or is being carried on:

The University of Arizona: A test to determine consumer taste preference for milk with different levels of butterfat and solids-not-fat.

Penn State: Long-term psychological motivation study to determine what it is that influences adolescents to stop consuming milk when they become young adults.

Oklahoma State: To determine what consumers consider proper curd size, salt content, acidity, and creaming for Cottage Cheese.

The University of West Virginia: To determine the potential of cocoa for breakfast as a means of increasing consumption of milk.

Ohio State: To find the correlation of community, type of grocery store, and size of dairy display case to sales.

University of Maryland: A study is going on to determine if giving away recipe leaflets on Cottage Cheese increases sales.

In Des Moines, Iowa, a study was made to determine how sour cream can be promoted, and this was repeated in Minneapolis recently, taking into consideration what was learned in Des Moines.

In New York State, at Washington State University, and the University of Idaho a study was made of the characteristics of home delivery customers and their consumption of dairy products, to determine if the industry should encourage delivery as a way of increasing sales.

The University of Idaho has just completed a 2-yr. study trying to determine how

local advertising and promotion affect sales of dairy products.

All of this research is only a part of the research that is being supported by the American Dairy Association in cooperation with various land grant colleges and other institutions, and in many of them the USDA is involved. In addition, the ADA has other merchandising-oriented research such as:

A study of family eating habits to determine how many dairy products are used by the average family and just how they are used.

Continuous use of Gallup-Robinson to determine the impact of its media advertising.

A study of National Analysts, Inc., to determine the Psychological Appeal of Butter.

Continuous pre-testing of ads to determine in advance their effect on sonsumers.

Some of the work that's being done independently by the Experiment Stations over the country consists of:

Studies of the sales of additional milk through vending machines; studies of methods used by distributors to increase milk sales; studies on the demand-price characteristics of milk; studies of the potential market for concentrated or other forms of fresh milk; studies of the consumption patterns and interrelationships between fresh and other forms of processed milk; studies of specific methods of merchandising dairy products.

Another important group of merchandising studies is being carried on by USDA in areas other than the dairy industry, the results of which contribute to the general knowledge of merchandising and can be used by the dairy industry. To mention only a few:

Effects of coupons and special offers on sales of butter, margarine, and salad and cooking oils; the results of displaying dates in different-sized packages; the results of different types of displays and islands in merchandising canned goods in supermarkets; the effects of different methods of pricing by multiple units on selling potatoes; effects of different types of displays and advertising techniques on sales of Washington State apples.

The list is long. This is not nearly all of the projects being worked on, but it gives you a good idea of the type of work that is being done.

I would like to make two value judgments on the projects mentioned.

First, the really important work in dairy research, and this is the research aimed at keeping the dairy industry a leader, the merchandising-oriented research, if you please, is being spearheaded by, is being done by, or being supported by trade organizations, such as ADA, or private industry.

Academic institutions are notably lacking in this type of research, and it is practically nonexistent in dairy science departments. The technical dairy people seem too interested in cows, and feeds, and pasteurizers, and trucks for hauling milk to be interested in this one thing that keeps the industry going—the consumer. The dairy industry can have the highest producing cows imaginable, and the finest plants, and the highest quality products, from the technician's point of view, but unless the consumer wants what the industry is producing, these other things are to no avail. I am afraid that in too many universities and experiment stations the consumer is forgotten. In the effort of the researchers to be professional, or academic; they have forgotten the cause for their existence, the consumer.

Secondly, I will take my colleague to task, the academic economist, who sometimes gets so engrossed in detailed nose-counting—so many dairies do this, so many do that, so many housewives buy this many, and so many that many—that he does not come to grips with the real problem, the problem of understanding the market and merchandising.

But, maybe I'm too harsh in my judgment, because of the third point in my discussion today, the differences between the way the results of merchandising research have to be interpreted and used and the way physical science research results can be interpreted and used. Maybe this transition between the two is too much to ask of people who have been trained in one way, in the physical science way, when trying to work in another area.

The basic difference comes in the fact that the experimental method is used in merchandising research, because it is the kind of research that the physical sciences have proven to be most valid . . . but merchandising research does not lend itself to this type of approach. The reason it does not is that the experiment is based on the assumption that all factors bearing on a particular system can be controlled during an experiment, with only the factor to be measured varying so that the results of its variation can be noted and measured; a refinement of this is a statistical method of design that eliminates statistically the effects of all factors but the one to be varied.

In any case, the experimental method assumes that all factors bearing on a system are known, and that their effect is always in the same direction at the same time . . . but, in merchandising the first pitfall is that not all factors that go into making a sale are known, and even if known, have been proven not to affect results in the same direction under apparently similar conditions. While this last point is too long to take up here, the net results of it are that at best only qualitative

judgments can be made from it, not quantitative; advertising and promotion—merchandising—is an art, not a science. This is not to say that merchandising can not make good use of research, but the results of the research have to be understood in the context of the problem involved.

The one last thought I would like to leave with you, as dairy science research people, is that for the good of the dairy industry more of your time, effort, and resources should be devoted to the consumer—what pleases him, and how to reach him—than to cows, feeds, bacteria, and plants.

MODERN CONCEPTS OF DAIRY PLANT ENGINEERING

A. L. Rippen
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Modern concepts of dairy plant engineering vary considerably among individuals. Mitten (7) has stated, "The challenge ahead in dairy engineering is one of integrating men, methods, and machines to yield the highest quality products at the lowest cost so that more people can enjoy Nature's most nearly perfect food in its various forms."

A. L. Rippen

Engineering developments of the last decade provide the dairy industry with new tools for processing, packaging, and handling high quality dairy products. The new equipment and facilities also create a dilemma for some plant operators. "Should we install remote operated valves and automatic controls?" is the type of question being raised as management and engineers attempt to forecast as accurately as possible the savings which may accrue from an installation of latest design.

A discussion of some of the features of modern plants will serve to illustrate certain principles in building design, plant layout, and materials handling.

PLANT SITE

The trend toward single-story highly productive plants which service many vehicles has increased the need for ample land area. Formerly, plant site was thought of in terms of square feet, whereas we now refer to the site in terms of acres. A good plant site is rectangular in shape and reasonably level, permitting traffic to move completely around the main building. The traffic pattern for the various functions of a plant should not interfere with other operations. For example, the receiving of supplies and other services should not be impeded or cause delays in other plant functions.

A modern concept for milk plants is to have some auxiliary services performed in buildings, or areas separate from the main plant (2). For instance, the paint shop and garage for truck maintenance is conveniently located a short distance from the main building. There is adequate parking area to accommodate the automobiles of employees, customers, visitors, and others.

Present and future availability of utilities including a municipal waste disposal system is very important. Although often located near the outskirts of larger towns and cities, management exercises extreme care in making certain that there is an adequate source of all necessary utilities.

THE DAIRY BUILDING

The modern plant is not only economically efficient in performance but attractive in appearance. Judicious use of glass blocks, glass, and colored panels in curtain walls which are blended harmoniously with brick or other building materials, including various metals, gives the plant a pleasing appearance.

Small plants are usually one-story; whereas, large plants are quite often two-story with offices, surge tanks, and perhaps some processing on the second floor. Whether a plant is single or multistory there are no more partitions than necessary and a minimum of load-bearing walls (1). Long, clear-span construction, with as few partitions and load-bearing walls as possible, constitutes the basic design to achieve greater flexibility with provision for future expansion. Floor area, although ample, is efficiently utilized. The clearance required between equipment and walls provides incentive for having no more partitions than necessary. Corridors and passageways are carefully scrutinized from the standpoint of their value to the plant and have been held to a minimum.

Exterior curtain or panel walls and precast concrete floor and roof systems are used quite extensively. Indications are that prefabricated construction economizes on building costs and will probably be used more in the future.

Relatively rapid changes in equipment, methods, and processes cause obsolescence to take its toll on buildings as well as equipment. A prevailing concept of building construction provides that the materials be moderately priced,

meet sanitary standards, and be durable, for low maintenance costs. Thought is given to constructing buildings to last 30 yr. instead of 50 yr., as practiced in the past (3). Concrete or light weight blocks having a glazed surface are used rather extensively. Plain blocks are sometimes coated with epoxy plastic paints or other materials which result in a hard finish. Several wall coatings can be applied with a paint spray-gun and in some cases one or two coats of lacquer are added to provide a hard, durable finish. Structural ceramic tile is still used for high quality interior walls. Therefore, many modern plants are built unhesitantly with tile. In processing areas, vitreous floor tile is quite commonly installed, using acid-resistant bed and joint materials. This type floor has a higher initial cost but is regarded as very serviceable (6).

Building space being at a premium results in some equipment being placed out-of-doors but serving its function as an integral part of the operation. Raw and pasteurized storage tanks, ice builders, and evaporative condensers are frequently found around the perimeter of the building or on the roof of modern dairy plants.

Ventilation systems are designed to change the air in processing rooms every 3 to 4 min. Condensation on ceilings is controlled by introducing outside air through a heater in cold weather, which absorbs much of the excess moisture found in the plant air. Critical processing rooms are slightly pressurized to about 1½ in. static water pressure through controlled intake and exhaust. With mechanical ventilating systems few windows, if any, are found in processing rooms. However, glass blocks are used to admit some natural light in many plants.

To assure an adequate amount of light for various operations requires proper use of artificial lighting. A committee of the Illuminating Engineering Society (9) has established recommended minimum foot-candle values for dairy plants, as shown in Table 1. These are somewhat higher than earlier recommendations. Bottle inspections and similar close work require individual investigation to determine the proper placement and quantity of light necessary to obtain satisfactory results.

TABLE 1

Recommended foot-candles provided by artificial light for various dairy plant areas and functions

Area or operation	Recommended foot-candles
Boiler room, compressor room	30
Bottle storage	30
Bottle sorting	50
Can washing	30
Cooling equipment—cabinet coolers, plate, etc.	30
Gauges	50
Laboratory	100
Control panels	50
Pasteurizers	30
Separators	30
Cold storage room	30
Thermometers	50
Receiving room	30
Scales	70
Offices	
Accounting, tabulating, machine operations	150
Filing, mail-sorting	100
Corridors, elevators, stairways	20
Exterior areas	
Loading and unloading platforms	20

PLANT LAYOUT AND MATERIALS HANDLING

A plant serves the needs of many phases of an operation including procurement, processing, sales and distribution, quality control, and record-keeping. The ideas of many individuals are considered in arriving at a plant layout which will function with maximum economy. Architects and engineers must know detailed procedures and methods to be used in processing and handling of products and supplies before making a layout of the modern plant (8).

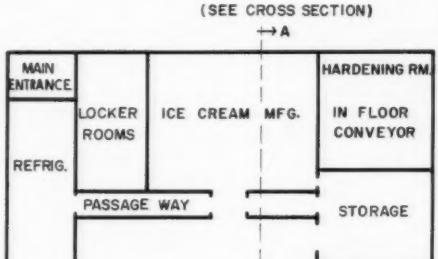

FIG. 1. First floor operational areas showing passageway (no scale).

A rather unique plan is used for distributing utilities in a new two-floor basementless plant. Figures 1 and 2 show the operational areas of the first and second floor of the plant and indicate the location of a utility corridor over a centralized passageway. Most utilities are efficiently distributed to operations on both floors directly from the corridor, as shown in Figure 3. To prevent uncomfortably high temperatures in the passageway, fire-resistant insulation has been placed above the removable ceiling sections. Electrical distribution panels

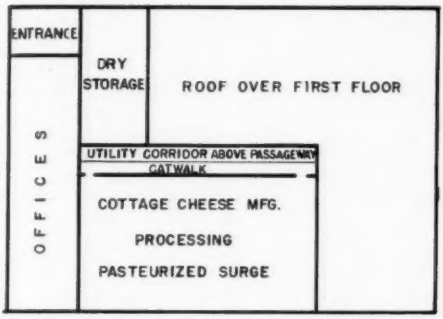

FIG. 2. Second floor areas showing utility corridor (no scale).

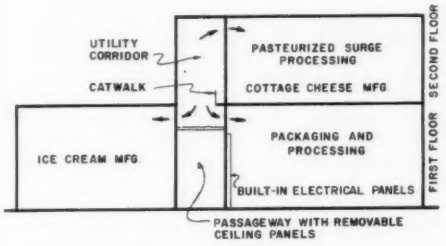

FIG. 3. Cross section A-A′ of a basementless plant with utilities originating at end of utility corridor (no scale).

are neatly recessed into the wall of the passageway and equipped with smooth metal doors.

Some plants have refrigeration, steam, compressed air, and water lines, as well as electrical services, enclosed inside a large utility duct located along a wall or above the ceiling. Regardless of location, the duct surfaces exposed to the rooms are neatly finished with removable panels for accessibility. In some cases stainless steel panels are used. Branches of all utilities are brought through the paneled sections at convenient points for easy connection to plant equipment. When the utilities originate in a basement, the piping is brought up through floor islands conveniently located for short-line connections to equipment. Floor islands are about 4 sq. ft. and 6 to 8 in. high. The islands are made of floor tile, stainless steel, or other suitable material.

Walking requires time, therefore, rooms and equipment are arranged to minimize walking for machine operators and supervisory personnel. The use of casers, case-handling equipment, and fillers are often so arranged that an operator is surrounded by machines to facilitate control of operations. Figures 4 and 5 illustrate the principle of an operator being centrally located among automatic packaging equipment for glass and paper. This arrangement has proven to be quite efficient in medium- and larger-size plants.

Hall (3) emphasizes the need to improve processing operations from an efficiency standpoint, with particular analysis and attention given to the methods used in furnishing supplies and services to all functions. Stressing the efficiency of the processing operation itself is not enough, unless adequate consideration has been given to all of the services and supplies required to perform the processing function.

The modern plant utilizes several different materials handling devices or systems. Pallets are often used to handle supplies in the central storage and operate in conjunction with conveyors having automatic discharge mechanisms which deposit the supplies at the point of use. Conveyors with special belt and wheel combinations provide adjustable pressure control between cartons assembled in live storage feeder systems to the filling machines. This method is used especially in supplying paper cartons for fillers. There is increased interest in the perimeter supply principle used in conjunction with live storage systems to increase the efficiency of machine operators. Mechanization of moving supplies from dry storage to point of use is considered a must in the modern dairy plant. Mechanical devices such as in-floor conveyors, casers, stackers, and pallets permit a man to handle small items in multiple units which increases the productivity per man hour. Using liquid sugar, liquid carton coating, and other items which can be handled in bulk increases efficiency when sufficient volume is involved.

Handling most dairy products themselves

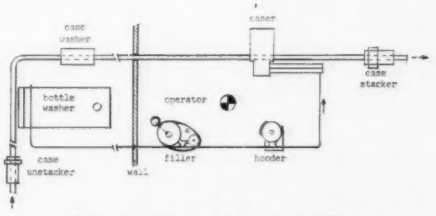

FIG. 4. Glass bottle operation.

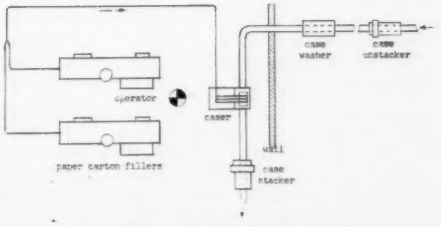

FIG. 5. Paper carton operation.

is not particularly difficult, because they can be pumped and piped relatively easily. Modern methods of cleaning-in-place make the sanitation problem less difficult. Distance is no longer a major factor in moving the product from one operation to another. Therefore, the plant layout now incorporates the most efficient combination of materials handling devices for furnishing supplies, with somewhat less emphasis on straight line flow of the product itself.

The air space in rooms with high ceilings is not wasted. This is particularly true in dry storage areas and is becoming more important in refrigerated storage rooms. The use of fork lifts and also single track conveying systems suspended near the ceiling makes product storage possible upwards of 8 ft. above the floor. Modern concepts of plant layout take advantage of the cube space which is available.

In-place-cleaning with welded or semipermanent lines has made it necessary for more preplanning of equipment arrangements and processing schedules than was required in the past when takedown sanitary lines were used. The opportunity to make quick temporary piping setups is restricted considerably, even by semipermanent CIP systems. Piping systems installed for CIP determines to a large extent where machines will be located (10). Like or similar pieces of equipment are grouped together for ease in making piping connections and to provide flexibility of product flow.

The growing use of relatively permanent CIP lines, spray cleaning of tanks and vats, and remote operated process valving is indicative of the present trend in dairy engineering. With better cleaning procedures, materials handling equipment, and instrumentation it is easier in some respects to modify and remodel an older plant than has been true in the past. However, space limitations and difficulties in obtaining efficient coordination of the many plant functions usually confront those attempting to remodel present facilities.

PLANT OPERATIONS

Concentration of control is a phrase we can use to describe a basic concept in dairy plant engineering from an operational standpoint. An operator's effectiveness is greatly extended through remote control of product flow and handling of supplies. More plant functions can be handled from a central control station than has ever been true in the past. Sanitation is of prime importance and must govern engineering features that can be employed.

Some modern plants have semigraphic illuminated panels at key process areas from which the flow of a variety of dairy products is automatically controlled. The system is so interlocked that the danger of intermixing products or intermixing product and cleaning solution is negligible. A Michigan dairy has installed remote-controlled air-operated sanitary valves in conjunction with three control panels and automatic CIP system (12). Each panel has a stainless steel housing with plastic facing, a process flow chart, selector switches, product location chart, and lighting fixtures. The panel identifies products in the various tanks, operation of some of the key units, and indicates product flow. Moving a selector switch on one of the panels will indicate the change on the other panels. Critical information is thus relayed to operators in different areas of the plant. Control of the CIP system is achieved with two program timers which are 30-function, 120-step rotary drum units. The drums have perforations into which plastic pins are inserted in a certain arrangement to produce the desired results. One of the timers controls the CIP unit while the other controls valve operations so as to attain maximum operating flexibility. Both of the timers are interlocked, so that they do not get out of phase. The system has four circuits for cleaning, which may be selected with the selector switch. Each circuit receives the proper programming of temperatures, times, detergent concentration, and rinse combinations which have been predetermined for the needs of that particular circuit.

It is important to realize that when a system has air-operated or similar valves it is usually necessary that automatic control of the in-place-cleaning system be used to adequately clean the lines and valves.

Although there is a gradual increase in the use of remote-controlled automatic CIP valves, a number of plant operators hold the concept of exploiting the use of new materials handling equipment before completely automating product flow. These plants with hand-operated valves use CIP methods of cleaning. Automation must produce a savings and return the original investment to owners preferably in no more than 3 yr. Therefore, in several instances management awaits more information on probable savings resulting from fully automated flow control.

Modern equipment provides greater capacity than ever before. For example, present models of high temperature–short time pasteurization units reach capacities upwards of 50,000 lb. of milk per hour. Large continuous processing units are making more efficient use of manpower as well as floor space. Three-tube ice cream freezers support the concept of package units which reduce the cost of installation.

A few small-volume plants find it possible and practical to operate the fluid milk department three days per week. Adequate storage capacity is necessary for the finished product; also, careful control of quality is essential. The three-day schedule permits workers to manufacture by-products on alternate days during the week. However, most plants, especially larger operations, find it more economical to operate five or six days a week several hours

a day, often involving two or more shifts. Increased volume and longer running time better utilize the fixed assets of production facilities.

Continuous flow processing, including the hardening of ice cream, makes it possible to reduce the amount of finished products in plant storage when trucks are loaded directly from the production line. In some cases, trucks are unloaded of empty cases and are immediately reloaded on one spot.

UTILITIES AND THEIR USE

Steam. Where processing temperatures are under 240° F., low pressure steam (less than 15 p.s.i.) has proven to be satisfactory and economical in several installations (4). A key factor is the possible reduction in labor costs when local regulations do not require a special stationary boiler operator. Low-pressure steam results in lower maintenance costs for valves, steam traps, and controls. A slightly lower fuel cost is another advantage of low-pressure steam.

Modern boilers convert oil or gas fuels to steam consistently with 75 to 80% efficiency. Steam is being better utilized through improved design of heat exchange equipment. Heat transfer surfaces are sometimes scraped mechanically to improve efficiency when high processing temperatures must be attained. With the advent of ultra-high processing temperatures, the purity and quality of steam generated for direct injection heating methods must, of necessity, be extremely high.

Refrigeration. Present refrigeration systems operate to a large degree automatically. Thermostats, time delay switches for automatic starting intervals of compressors, pressure controls, and the like provide the means for maintaining proper refrigerated storage and product cooling temperatures. High-speed compressors are used to economize on floor space. Because of smaller component parts the high-speed machines are easier to repair than older style units. Modern low-temperature refrigeration systems use two-stage compression to attain greater economy. Cooling towers or evaporative condensers reduce water requirements as much as 90 to 95%, and are used extensively.

Refrigerated rooms for temperatures of 34 to 40° F. are constructed without floor insulation when the floor slab is on the ground. Heat gain through the floor is negligible when compared with the walls and ceiling (5). Therefore, the cost of floor insulation is not ordinarily justified for refrigerated milk storage rooms.

Compressed air. Compressed air is a very versatile source of energy. Its quick response to valving changes and ease of regulation make it exceptionally well-suited for automatic control systems. Much new equipment developed for the dairy industry makes extensive use of compressed air. Modern plants have sizable compressed air systems which are frequently equipped with after-coolers for moisture removal.

PLANTS OF THE FUTURE

It is reasonable to assume that future engineering and processing developments will eventually result in plants which are extremely efficient in the production of high quality dairy products. Two hundred gallons of milk handled per man-hour in a fluid milk operation could be commonplace compared with about 80 to 90 gal. per man-hour in some of our better present-day plants.

Tomorrow's milk plant operations may conceivably be controlled from a master control center, as some have suggested (11). The master control center would monitor the overall functions of the plant and would accumulate, segregate, audit, and issue pertinent information to subcontrol stations. Such an operation is obviously some years away, but it does serve as a guide to modern trends in the thinking of engineers, physicists, chemists, progressive dairymen, and others.

CONCLUSION

In conclusion, it should be mentioned that we are in a period of transition and are achieving refinements and standardization of automated systems which will be acceptable to all segments of the dairy industry. Some degree of automation has been in common practice for several years. But, not all firms will likely adopt extensive automation until the advantages for them become clearly evident. Several managers and engineers are exerting a coordinated effort in planning the future of their organization with the philosophy of being neither the first nor the last to automate.

ACKNOWLEDGMENTS

The author expresses thanks to the plant managers and engineers who offered excellent suggestions and for making their operations available for study in the preparation of this article. Special thanks are due Professor C. W. Hall and Professor T. I. Hedrick, Michigan State University, for their helpful ideas. Credit is due the Cooperative Extension Service and USDA for financial support through the Market Agency Education Program.

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ASSOCIATION AFFAIRS

NOMINATIONS FOR OFFICERS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

K. L. TURK, Chairman Nominating Committee
Cornell University, Ithaca, New York

The Nominating Committee of the American Dairy Science Association has selected persons for the offices of Vice-President and Directors of the Association. To balance the Board of Directors for the three educational branches of the Association, it will be necessary this year to elect two directors from the Extension Section. The following nominees are submitted:

VICE-PRESIDENT

George Hyatt, Jr.

I. W. Rupel

Biographies

GEORGE HYATT, JR.

George Hyatt, Jr. was born December 14, 1914, in Toledo, Ohio. He is Professor and Head of the Department of Animal Industry at North Carolina State College, Raleigh. He earned the B.S. degree at Michigan State University in 1937, the M.S. degree at Rutgers University in 1941, and the Ph.D. at the University of Wisconsin in 1961.

He has held the following positions:

1937-39—Milk Inspector, City of Detroit
1939-41—Research Fellow, Rutgers University
1941-43—Assistant Extension Dairyman, West Virginia University
1943-46—Extension Dairyman, University of Maryland
1946-51—Associate Professor and Professor Dairy Husbandry, West Virginia University
1951-52—Farm Manager and Editorial Adviser, Hoard's Dairyman
1952-57—Head, Dairy Extension, North Carolina State College
1957-58—Kellogg Foundation Scholarship, University of Wisconsin
1958 to date—Professor and Head, Department of Animal Industry, North Carolina State College

DIRECTOR

Raymond Albrectsen

Robert B. Redfern

DIRECTOR

Charles D. McGrew

C. W. Nibler

Academic and Professional Honors: Member of American Society of Animal Production; Sigma Xi, Phi Kappa Phi; Listed in Who's Who in American Agriculture; American Men of Science.

A.D.S.A. Activities: Board of Directors. Secretary, Vice-Chairman, and Chairman, Production Section. Has served on following committees: dairy records, membership, DeLaval extension award, special association committee to study awards program, public health.

Other Information: Official classifier, American Jersey Cattle Club.

I. W. RUPEL

I. W. Rupel was born May 3, 1900, in Walkerton, Indiana. He is Professor and Head of the Department of Dairy Science at Texas A & M. College, College Station. He earned the B.S. at the University of Illinois in 1923, the M.S. and the Ph.D. degrees at the Univer-

sity of Wisconsin in 1924 and 1932, respectively.

He has held the following positions:

1924-29—Instructor in Animal Husbandry (Dairy Section), University of Wisconsin
January-June, 1930—Exchange Professor of Agriculture, University of Hawaii
1930-45—Assistant and Associate Professor of Animal and Dairy Husbandry, University of Wisconsin
1945 to date—Professor and Head, Department of Dairy Science, Texas A & M College

Academic and Professional Honors: Fellow, American Association for the Advancement of Science; Fellow, Texas Academy of Science, member Sigma Xi, Phi Sigma, Phi Kappa Phi, and Alpha Zeta. Member American Society for Animal Production, 32 yr.; Editorial Board, Journal Animal Science 1951-54; Chairman, Dairy Section, 1 yr. and member Committee on Fellows 1957-59. Member United States Livestock Sanitary Association 1950-; member Society for Experimental Biology and Medicine 12 yr. Official judge of livestock, National Fair, Guatemala, 1941. Official classifier, American Jersey Cattle Club 16 yr. Member north central committee on dairy housing 1942-45. Advisory delegate to 15th International Dairy Congress (London, 1959); life member Dairy Shrine Club. Director American Dairy Association of Texas 15 yr.

A.D.S.A. Activities: Director, American Dairy Science Association 1955-58; Associate Editor, Journal of Dairy Science 1941-51; Secretary, Production Section 1937; member Curriculum Committee 1940-42; Chairman, Graduate section 1951-53; Nominating committee 1947; Resolutions committee 1952-54, Chairman 1954; Rules committee National Collegiate Dairy Cattle Judging Contests, 17 yr., Chairman 11 yr.; Constitution Revision committee 1954; Policy committee (advisory to the Executive Board) 1958-; Public Health committee 1960-; Director Southern Division 3 yr.; Secretary-Treasurer Southern Division 1961-62.

RAYMOND ALBRECTSEN

Raymond Albrectsen was born October 12, 1904, in Chicago, Illinois. He is Professor of Animal Husbandry, at Cornell University, Ithaca, New York. He attended Pennsylvania State College during 1926 to 1928. He earned the B.S. and M.S. degrees at Cornell University in 1930 and 1931, respectively.

He has held the following positions:

1930-31—Assistant in Dairy Husbandry, Cornell University
1931-37—Operated dairy farm
1937-39—Extension Instructor, Dairy Husbandry, Cornell University (part time)
1939-44—Extension Instructor, Dairy Husbandry, Cornell University
1944-47—Extension Assistant Professor in Dairy Husbandry, Cornell University
1947-49—Associate Professor in Dairy Husbandry, Cornell University
1949-58—Professor of Animal Husbandry, Cornell University
1958 to date—Professor, Project Leader, Animal Husbandry Extension, Cornell University (Animal & Dairy Husbandry)

Academic and Professional Honors: DeLaval Extension Dairyman Award, 1959; Superior Service Award, U. S. Department of Agriculture, 1955; Member of Epsilon Sigma Phi.

A.D.S.A. Activities: Secretary 1949, Vice-Chairman 1950, and Chairman 1951 Extension Section; at various times has been member and chairman of the breeding committee; secretary, Breeds Relations Committee; chairman and secretary, Type Committee; chairman, Planning Committee for establishing Extension Dairyman's Award; chairman DeLaval Extension Dairyman Award Committee; Nominating Committee 1960.

Other Information: Member of the advisory work group to Division of Dairy Herd Improvement Investigations, USDA; successful breeder of Holstein-Friesian cattle. Operates 90-cow herd of registered cattle; first president of the first artificial breeding cooperative in New York State; assisted in the organization of New York Artificial Breeders' Cooperative and National Association of Artificial Breeders; served as consultant to one of the largest dairy operations in Argentina in October, 1960.

ROBERT B. REDFERN

Robert B. Redfern was born May 25, 1919, at Mt. Croghan, South Carolina. He is Associate Professor of Animal Industry and Dairy Manufacturing Extension Specialist, North Carolina State College, Raleigh. He earned the B.S. degree at Clemson A & M College in 1941, and the M.S. and Ph.D. degrees at North Carolina State College in 1949 and 1951, respectively.

He has held the following positions:

1941-46—U. S. Armed Forces
1947-52—Research Assistant, North Carolina State College
1952-59—Assistant Professor of Animal Industry, North Carolina State College
1960 to date—Associate Professor of Animal Industry, North Carolina State College

Academic and Professional Honors: Member of Sigma Xi; Alpha Tau Alpha; Who's Who in American Education; American Men of Science.

A.D.S.A. Activities: 1960-61 Chairman, Dairy Manufacturing Extension Section. Has served on Membership Committee.

Other Information: Secretary-Treasurer, North Carolina Dairy Industry Promotion Committee; Secretary, North Carolina Dairy Technology Society; Adviser to North Carolina Dairy Products Association.

CHARLES D. MC GREW

Charles D. McGrew was born April 21, 1905, at Kent, Ohio. He is Extension Specialist in Dairy Science at the Ohio State University, Columbus. He earned the B.S. degree at The Ohio State University in 1928 and the M.S. degree at Cornell University in 1949. He attended Colorado State University in 1938 and in 1953, and also the University of Wisconsin in 1954 and Cornell University in 1958.

He has held the following positions:

1931-46—County Agricultural Agent, Ohio
1947 to date—Extension Dairy Specialist, Ohio State University

Academic and Professional Honors: Alpha Zeta, Gamma Sigma Delta, Epsilon Sigma Phi.

A.D.S.A. Activities: 1961 Secretary, Extension Section. Has been member and Chairman of Dairy Cattle Breeding and Dairy Cattle Type committees.

C. W. NIBLER

C. W. Nibler was born March 6, 1905, in Boise, Idaho. He is Extension Dairyman at the University of Nebraska, Lincoln. He earned the B.S. degree at the University of Idaho in 1928 and the M.A. degree at the University of Missouri in 1929. He attended the Extension Workers' Course during the summer of 1936 at Colorado State University.

He has held the following positions:

1929-32—Instructor in Dairy Husbandry, University of Nebraska
1932-36—County Agricultural Agent, Kimball County, Nebraska
1936-45—County Agricultural Agent, Scottsbluff County, Nebraska
1945 to date—Extension Dairyman, University of Nebraska

Academic and Professional Honors: Distinguished Service Award from University of Nebraska; Member of Alpha Zeta; Gamma Sigma Delta; Sigma Xi; Epsilon Sigma Phi.

A.D.S.A. Activities: 1958-60 Secretary, Vice-Chairman, and Chairman, Extension Section; 1949-50 Chairman, 4-H Club Committee; 1957-58, Resolutions Committee; 1957-58, DeLaval Awards Committee; 1958-59, Nominating Committee; 1959-60, Program Committee.

PROGRAM

Fifty-Sixth Annual Meeting

of the

AMERICAN DAIRY SCIENCE ASSOCIATION

University of Wisconsin

Madison

June 11–14, 1961

PROGRAM COMMITTEE

G. W. Trimberger, New York, Chairman
A. C. Fay, Florida, Ex Officio
R. P. Niedermeier, Wisconsin
S. D. Musgrave, Oklahoma
Burdet Heinemann, Missouri
M. E. Senger, North Carolina

GENERAL PROGRAM

Friday, June 9

1:00 P.M. **Meeting of Executive Board Committees,** Wisconsin Center

Saturday, June 10

9:00 A.M. **Meeting of Executive Board,** Wisconsin Center

Sunday, June 11

9:00 A.M. **Meeting of Executive Board,** Wisconsin Center

1:30–10:00 P.M.
Registration, Babcock Hall

7:00 P.M. **Informal Get-Together,** Elm Drive Commons

Monday, June 12

8:00 A.M.–9:00 P.M.
Registration, Babcock Hall

9:00–11:45 A.M.
Opening Session, Wisconsin Union Theater
E. E. Heizer, Chairman, Department of Dairy Husbandry, University of Wisconsin, Presiding

National Anthem, D. W. Gilbert, School of Music, University of Wisconsin

Invocation, Reverend Sig G. Sandrock, Midvale Community Lutheran Church, Madison

Welcome, R. K. Froker, Dean, College of Agriculture, University of Wisconsin

Presidential Address, R. E. Hodgson, Director, Animal Husbandry Research Division, USDA

Guest Speaker, C. A. Elvehjem, President, University of Wisconsin, "Some Significant Dates in the History of Dairy Research"

10:30–11:45 A.M.

Program Sponsored by Education Committee
Wisconsin Union Theater

Changes Which May Be Forced upon Us by Trends in College Enrollment

Changes in College Policy. A. E. Darlow, Vice President and Dean of Agriculture, Oklahoma State University

Changes in Dairy Manufacturing Education. B. L. Herrington, Chairman A.D.S.A. Education Committee, Cornell University

Changes in Dairy Production Education. George Hyatt, Jr., Chairman, Department of Animal Industry, North Carolina State College

4:30 P.M. **Past Presidents' Program and Dinner**
Wisconsin Union, Round Table Room

7:30 P.M. **The Mazur Polish Dancers,** Wisconsin Union Theater

Tuesday, June 13

8:00 P.M. **Recognition Program and Presentation of Awards**
Wisconsin Union Theater

8:00 P.M. **Teenage Dance,** Wisconsin Union, Tripp Commons

Wednesday, June 14

10:00–11:00 A.M.

Invitational Paper: Field Problems with Pesticide Residues.
H. E. O. Heinemann, Pet Milk Company

Discussant: J. E. Casida, Department of Entomology, University of Wisconsin
Agricultural Hall Auditorium

11:00 A.M.–12:30 P.M.

General Session Devoted to Association Affairs
Agricultural Hall Auditorium

4:00 P.M. Programs Conclude

MANUFACTURING SECTION

Monday, June 12

9:00–10:30 A.M.

Opening Session
Wisconsin Union Theater

10:30–11:45 A.M.

Program Sponsored by Education Committee
Wisconsin Union Theater

1:30– 4:30 P.M.

Section A. **Market Milk**
Babcock Hall, Room 205

Section B. **Concentrated and Dried Products**
Biochemistry, Room 101

Section C. **Cultures**
Moore Hall, Room 102

4:30– 5:00 P.M.

Manufacturing Section Business Meeting
Babcock Hall, Room 205

Tuesday, June 13

8:30–10:45 A.M.

Section A. **Dairy Bacteriology**
Babcock Hall, Room 205

Section B. **Milk Proteins**
Biochemistry, Room 101

Section C. **Dairy Manufacturing Extension**
Moore Hall, Room 102

11:00–12:00 M.

Manufacturing Section Business Meeting
Babcock Hall, Room 205

1:30– 4:30 P.M.

Section A. **Dairy Chemistry**
Babcock Hall, Room 205

Section B. **Cheese and Butter**
Biochemistry, Room 101

Section C. **Dairy Manufacturing Extension**
Moore Hall, Room 102

4:30– 5:30 P.M.

Informal Meeting for Discussion of Cheese Cultures
Babcock Hall, Room 121

Wednesday, June 14

8:00– 9.45 A.M.
All Sections
Symposium: Protein Methodology
Babcock Hall, Room 205

10:00–11:00 A.M.
Joint with Production and Extension Sections
Invitational paper: Field Problems with Pesticide Residues
H. E. O. Heinemann, Pet Milk Company

Discussant: J. E. Casida, Department of Entomology, University of Wisconsin
Agricultural Hall Auditorium

11:00 A.M.–12:30 P.M.
General Session Devoted to Association Affairs
Agricultural Hall Auditorium

1:30– 3:30 P.M.
Section A. **Milk Lipids**
Babcock Hall, Room 205

Section B. **Cottage Cheese and Ice Cream**
Biochemistry, Room 101

PRODUCTION SECTION

Monday, June 12

9:00–10:30 A.M.
Opening Session
Wisconsin Union Theater

10:30–11:45 A.M.
Program Sponsored by Education Committee
Wisconsin Union Theater

1:30– 4:30 P.M.
Section A. **Feed Utilization**
Commerce Auditorium

Section B. **Nutrition**
Commerce, Room 22

Section C. **Artificial Breeding**
Commerce, Room 19

4:30– 5:30 P.M.
Joint with Extension Section
Meeting of Superintendents of Official Testing
Bacteriology Auditorium

Tuesday, June 13

8:30–10:45 A.M.

Section A. **Calf Nutrition**
Commerce Room 19

Section B. **Reproductive Physiology**
Commerce, Room 22

Section C. **Genetics**
Commerce Auditorium

11:00–12:00 M.

Production Section Business Meeting
Commerce Auditorium

1:30– 4:30 P.M.

Section A. **Joint with Extension Section**

Symposium: A Modern Dairy Cattle Feeding Program
Agricultural Hall Auditorium

Section B. **Physiology**
Commerce, Room 19

Section C. **Reproductive Physiology and Genetics**
Commerce Auditorium

4:30– 5:30 P.M.

Joint with Extension Section

Joint Committee Reports
Agricultural Hall Auditorium
Dairy Cattle Breeding. R. E. Corbett, Maine
Dairy Cattle Health. V. R. Smith, Arizona
Dairy Cattle Type. R. Wayne, Minnesota

Wednesday, June 14

8:00– 9:45 A.M.

Section A. **Calf Nutrition**
Commerce, Room 19

Section B. Joint with Extension Section

Symposium: Recent Advances in Reproductive Efficiency
Commerce Auditorium

10:00–11:00 A.M.

Joint with Extension and Manufacturing Sections

Invitational paper: Field Problems with Pesticide Residues
H. E. O. Heinemann, Pet Milk Company

Discussant: J. E. Casida, Department of Entomology, University Wisconsin
Agricultural Hall Auditorium

11:00 A.M.–12:30 P.M.
General Session Devoted to Association Affairs
Agricultural Hall Auditorium

1:30– 3:30 P.M.
Section A. **Milk Production**
Commerce Auditorium

Section B. **Rumen Physiology**
Commerce, Room 22

Section C. **Forages**
Commerce, Room 19

EXTENSION SECTION

Monday, June 12

9:00–10:30 A.M.
Opening Session
Wisconsin Union Theater

10:30–11:45 A.M.
Program Sponsored by Education Committee
Wisconsin Union Theater

1:30– 4:30 P.M.
Teaching Methods
Bacteriology Auditorium

4:30– 5:30 P.M.
Joint with Production Section
Meeting of Superintendents of Official Testing
Bacteriology Auditorium

Tuesday, June 13

8:30–10:15 A.M.
4-H Club Program
Bacteriology Auditorium

10:30–12:00 M.
Extension Section Business Meeting
Bacteriology Auditorium

1:30– 4:30 P.M.
Joint with Production Section

Symposium: A Modern Dairy Cattle Feeding Program
Agricultural Hall Auditorium

4:30– 5:30 P.M.

Joint with Production Section

Joint Committee Reports
Agricultural Hall Auditorium
Dairy Cattle Breeding. R. E. Corbett, Maine
Dairy Cattle Health. V. R. Smith, Arizona
Dairy Cattle Type. R. Wayne, Minnesota

Wednesday, June 14

8:00–9:45 A.M.

Joint with Production Section
Symposium: Recent Advances in Reproductive Efficiency
Commerce Auditorium

10:00–11:00 A.M.

Joint with Production and Manufacturing Sections
Invitational paper: Field Problems with Pesticide Residues
H. E. O. Heinemann, Pet Milk Company

Discussant: J. E. Casida, Department of Entomology, University of Wisconsin
Agricultural Hall Auditorium

11:00 A.M.–12:30 P.M.

General Session Devoted to Association Affairs
Agricultural Hall Auditorium

1:30– 4:30 P.M.

Production Testing and the New Proved Sire Report
Bacteriology Auditorium

STUDENT BRANCH SECTION

Sunday, June 11

1:30–10:00 P.M.

Registration, Babcock Hall

3:00 P.M. **Executive Committee Meeting**
Dairy Cattle Center, Room 202

7:00 P.M. **Informal Mixer**
Elm Drive Dormitory B.

Monday, June 12

Student affiliates are encouraged to participate in the A.D.S.A. program during open periods.

9:00–10:30 A.M.

Opening Session
Wisconsin Union Theater

10:30–11:45 A.M.

Program Sponsored by Education Committee
Wisconsin Union Theater

12:00 M.– 1:30 P.M.

Luncheon with A.D.S.A. Members and A.D.A. Dairy Princess
Wisconsin Union

1:30– 2:30 P.M.

Student Branch Opening Session
Wisconsin Union

4:30– 5:30 P.M.

Student Branch Meeting

Summary of Outstanding Chapter Activities
Joseph Lineweaver, 1st Vice President
Chapter Highlights by Candidates for National Office

Tuesday, June 13

12:15– 1:45 P.M.

Luncheon with A.D.S.A. Members
Wisconsin Union

3:30– 5:30 P.M.

Symposium: Selecting a Career in the Dairy Industry

Moderator: G. M. Trout, Michigan State University
Dairy Cattle Center, Room 201

Foreign Service. D. L. Hume, Director of the Dairy and Poultry Division, Foreign Agricultural Service, USDA, Beltsville, Maryland

Education and Research. Earl Weaver, Recipient 1960 A.D.S.A. Teaching Award, Michigan State University, East Lansing, Michigan

Production. H. A. Herman, Executive Secretary of the National Association of Artificial Breeders, Columbia, Missouri

Manufacturing. H. F. Judkins, Secretary-Treasurer, American Dairy Science Association, White Plains, New York

Wednesday, June 14

8:00–10:15 A.M.

General Business Meeting of the Student Branch
Dairy Cattle Center, Room 201

Election of officers
Discussion of Exhibits and Chapter Activities

10:30–12:00 M.
General Session Devoted to Association Affairs
Agricultural Hall Auditorium

1:30– 3:30 P.M.
Meeting of Old and New Executive Committees with Advisors
Dairy Cattle Center, Room 202

1:30– 2:00 P.M.
Film, Careers in Agriculture
Dairy Cattle Center, Room 201

ENTERTAINMENT

General Program

Sunday, June 11 — 7:00 P.M.—**Informal Get-Together**
Elm Drive Commons

Monday, June 12 — 4:30 P.M.—**Past Presidents' Program and Dinner**
Wisconsin Union, Round Table Room

7:30 P.M.—**The Mazur Polish Dancers**
Wisconsin Union Theater

Tuesday, June 13 — 8:00 P.M.—**Recognition Program and Presentation of Awards**
Wisconsin Union Theater

8:00 P.M.—**Teenage Dance**
Wisconsin Union, Tripp Commons

Women's Program

(See Separate Program)

Monday, June 12 — 9:30–11:30 A.M.—**Informal Reception**
Home of President and Mrs. C. A. Elvehjem, 130 North Prospect Avenue

1:30– 4:30 P.M.—**Tour of Little Norway and New Glarus Lace Factory**

7:30 P.M.—**The Mazur Polish Dancers**
Wisconsin Union Theater

Tuesday, June 13 10:00–11:00 A.M.—**Visit Wisconsin Historical Museum**

12:30– 2:30 P.M.—**Luncheon and Program**
Wisconsin Union

8:00 P.M.—**Recognition Program and Presentation of Awards**
Wisconsin Union Theater

Wednesday, June 14 9:30–11:00 A.M.—**Social Hour**
Elm Drive Commons, Hide-Away

Children's Program
(See Separate Program)
Children 4–5 Years

(All activities for this age group will be at First Congregational Church, 1609 University Avenue)

Monday, June 12 9:00–11:30 A.M.—**Supervised Care**

1:00– 5:00 P.M.—**Supervised Care**

Tuesday, June 13 9:30 A.M.–2:30 P.M.—**Supervised Care including Lunch**

Wednesday, June 14 9:00–11:30 A.M.—**Supervised Care**
Children 6–9 Years

Monday, June 12 9:00–11:45 A.M.—**Supervised Activities**
Elm Drive Dormitory B

1:00– 4:45 P.M.—**Tour of Gardner Bakery and Truax Field**
Assemble Elm Drive Dormitory B

Tuesday, June 13 9.00–10:30 A.M.—**Supervised Activities**
Elm Drive Dormitory B

10:30 A.M.–2:30 P.M.—**Trip to Villas Park Zoo**
(Including Lunch)
Assemble Elm Drive Dormitory B

Wednesday, June 14 9.00–11:30 A.M.—**Supervised Activities**
Elm Drive Dormitory B
Children 10 and older

Monday, June 12 9:00–11:45 A.M.—**Tour of Verona School Forest**
Assemble Elm Drive Dormitory C

1:00– 4:45 P.M.—**Supervised Activities**
Elm Drive Dormitory C

Tuesday, June 13 9:00 A.M.–2:30 P.M.—Children 10–14 years, **Trip to Truax Field and Vilas Park Zoo Including Lunch**

Children 14 and older, **Trip to Truax Field and Oscar Mayer Packing Plant**

Assemble Elm Drive Dormitory C

Wednesday, June 14 9:00–11:30 A.M.—**Hike to Picnic Point**

MANUFACTURING SECTION

Monday, June 12

9:00–10:30 A.M.
Opening Session
Wisconsin Union Theater

10:30–11:45 A.M.
Program Sponsored by Education Committee
Wisconsin Union Theater

1:30–4:30 P.M.
Section A. **Market Milk**
B. Heinemann, Chairman
Babcock Hall, Room 205

M1. The evaluation of filling operations. R. B. Maxcy, University of Nebraska.

M2. Effect of chlorinated-detergent solution temperatures on the sanitary condition of a C-I-P farm milk pipeline. B. H. Snudden, H. E. Calbert, and W. C. Frazier, University of Wisconsin.

M3. Some detergency effects of chlorinated trisodium phosphate on milk protein solids. E. P. Merrill, S. T. Bass, and J. M. Jensen, Michigan State University.

M4. Establishing a homogeneous taste panel. T. J. Mucha and K. E. Nelson, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

M5. Statistical correlation between consumer reaction and organoleptic analysis of feed-flavored milk. T. J. Colson and R. Bassette, Kansas State University.

M6. Studies on flavor components from Ladino clover. A. E. Woods, L. W. Aurand, and W. M. Roberts, North Carolina State College.

M7. The mechanism of spontaneous peroxidation in milk. G. J. Smith and W. L. Dunkley, University of California.

M8. The effectiveness of nitrogen and sulfur chelating compounds in inhibiting the development of oxidized flavor in milk. P. F. Pierpont, C. M. Stine, and G. M. Trout, Michigan State University.

M9. Flavors imparted to dairy products by derivatives of phenol. J. A. Schlegel and F. J. Babel, Purdue University.

M10. A market survey of chip dips. G. C. Walter, C. M. Stine, and L. G. Harmon, Michigan State University.

M11. Effect of previous heat treatment and added solids-not-fat on the body of cultured cream. E. S. Guthrie, Cornell University.

M12. An investigation of an abnormal freezing characteristic of some milks. J. S. E. Sargent, A. G. Leggatt, D. R. Arnott, and D. A. Biggs, Ontario Agricultural College, Guelph, Ontario, Canada.

1:30– 4:30 P.M.

Section B. **Concentrated Dried Products**

D. M. Graham, Chairman

Biochemistry, Room 101

M13. Production of sulfur compounds in skimmilk heated by direct steam injection. C. W. Dill, W. M. Roberts, and L. W. Aurand, North Carolina State College.

M14. Effect of heat treatment on the viscosity of model milk systems. G. A. Muck and J. Tobias, University of Illinois.

M15. Sanitary aspects of condensers in milk plants. A. H. Rishoi, H. C. Jackson, and R. A. Marquardt, University of Wisconsin.

M16. Size of particles in sterile concentrated milk as related to concentration. H. K. Wilson, U. Yoshino, and E. O. Herreid, University of Illinois.

M17. Effect of homogenization on the oxidative stability of fat in sterilized milk. E. O. Herreid and H. K. Wilson, University of Illinois.

M18. Contact angle measurements of milk constituents and milk powder. C. H. Pyne and S. T. Coulter, University of Minnesota.

M19. The specific surface areas of milk powders produced by various drying methods. N. Howard, K. K. Fox, V. H. Holsinger, and M. J. Pallansch, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

M20. Direct recovery of added ketones from foam-dried whole milk. F. E. Kurtz, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

M21. Direct recovery of off flavor compounds from foam dried whole milk. F. E. Kurtz, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

M22. The inactivation and reactivation of xanthine oxidase in processing foam dried milk. G. R. Greenbank and M. J. Pallansch, Eastern Utillization Research and Development Division, USDA, Washington, D. C.

M23. The progress of oxidation in the milk powder granule. G. R. Greenbank and M. J. Pallansch, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

M24. Effect of solid-fat/liquid-fat ratio upon the sinkability of dried whole milk. W. E. Nelson and W. C. Winder, University of Wisconsin.

1:30– 4:30 P.M.

Section C. **Cultures**
M. L. Speck, Chairman
Moore Hall, Room 102

M25. Identification of substances in milk cultures of *Pseudomonas fluorescens* which stimulate lactic starter cultures. J. A. Koburger and T. J. Claydon, Kansas State University.

M26. Biochemical factors involved in symbiotic relationships among lactic streptococci. R. S. Dahiya and M. L. Speck, North Carolina State College.

M27. Effect of combining single-strain cultures as cheese starter on bitter flavor in Cheddar cheese at six months of age. D. B. Emmons, W. A. McGugan, J. A. Elliott, Dairy Technology Research Institute, and Pamela M. Morse, Statistical Research Service, Research Branch, Canada Department of Agriculture, Ottawa, Canada.

M28. Lactic acid and titratable acidity relationships in cultures and cultured products. W. J. Harper and H. E. Randolph, Ohio State University.

M29. The effect of some calcium-binding agents on the development of bacteriophage and growth of lactic streptococci in milk. V. W. Kadis and F. J. Babel, Purdue University.

M30. The accelerated recovery of dry starter cultures in milk fortified with pancreas extract. J. A. Koburger and M. L. Speck, North Carolina State College.

M31. Studies on the mechanism of diacetyl synthesis by *Streptococcus diacetilactis*. W. E. Sandine, E. W. Seitz, P. R. Elliker, and E. A. Day, Oregon Agricultural Experiment Station.

M32. Studies on factors affecting diacetyl production by *Streptococcus diacetilactis*. E. W. Seitz, W. E. Sandine, E. A. Day, and P. R. Elliker, Oregon Agricultural Experiment Station.

M33. Failure in production of acetoin and gas by cultures of *Streptococcus diacetilactis*. E. B. Collins and R. J. Harvey, University of California.

M34. Effect of lactose fortification on body, flavor, and biacetyl and acetoin content of cultured skimmilk. R. D. Marshall, C. M. Stine, and J. R. Brunner, Michigan State University.

M35. Destruction of penicillin during the Cottage cheese making process. K. L. Smith and J. E. Edmondson, University of Missouri.

4:30–5:00 P.M.

Manufacturing Section Business Meeting
B. Heinemann, Chairman
Babcock Hall, Room 205

Tuesday, June 13

8:30–10:45 A.M.

Section A. **Dairy Bacteriology**
M. L. Speck, Chairman
Babcock Hall, Room 205

M36. Is incubation for seven days at 5° C. adequate for enumerating psychrophilic bacteria in milk? W. E. Glenn, University of Kentucky.

M37. Comparison between Standard Methods procedure and a surface plate method for estimating psychrophilic bacteria in milk. J. D. Punch and J. C. Olson, Jr., University of Minnesota.

M38. The effect of penicillin in milk on the growth and survival of *Streptococcus aureus* during the manufacture and curing of Cheddar and Colby cheese. J. J. Jezeski, H. A. Morris, E. A. Zottola, E. George, and F. F. Busta, University of Minnesota.

M39. Effect of sodium chloride concentration in an agar medium on growth of heat-shocked *Staphylococcus aureus* Strain 196E. F. F. Busta and J. J. Jezeski, University of Minnesota.

M40. Preliminary observations on population levels of pure cultures of psychrophilic bacteria necessary to induce flavor or physical change in pasteurized milk. J. D. Punch, J. C. Olson, Jr., and E. L. Thomas, University of Minnesota.

M41. A study of inhibition of microbial lipases by antibiotics. R. C. Chandan, M. G. Carrancedo, and K. M. Shahani, University of Nebraska.

M42. Sensitivity of sugar metabolism of *Saccharomyces cerevisiae* to antifungal agents. M. G. Carrancedo, K. M. Shahani, and J. R. Vakil, University of Nebraska.

M43. The incidence and nature of the spore-forming microorganisms in the Ohio milk supply. J. H. Martin, D. P. Stahly, and W. J. Harper, Ohio State University.

M44. Development of *Clostridium botulinum* spores in sterile milk. O. W. Kaufman and A. R. Brillaud, Michigan State University.

8:30–10:45 A.M.

Section B. **Milk Proteins**

C. W. Gehrke, Chairman

Biochemistry, Room 101

M45. A simplified direct nesslerization procedure for total nitrogen in milk and cheese extracts. W. K. Stone, M. C. Conner, and N. R. Thompson, Virginia Polytechnic Institute.

M46. The choice of a primary standard for the determination of milk proteins by the dye-binding method. U. S. Ashworth, Washington State University.

M47. A simple quantitative method for the determination of β-lactoglobulin by an immunological procedure. B. L. Larson and Judith M. Twarog, University of Illinois.

M48. Observations on the paper electrophoresis of casein. L. M. Libbey and U. S. Ashworth, Washington State University.

M49. Dephosphorization of casein in solution and in milk by heat treatment. J. Belec and R. Jenness, University of Minnesota.

M50. The isolation of λ-casein from concentrated TCA-urea solutions. H. E. Swaisgood and J. R. Brunner, Michigan State University.

M51. Molecular structural studies on casein. II. W. G. Godbey and C. W. Gehrke, University of Missouri.

M52. Heat denaturation of β-lactoglobulins A and B. Patricia Gough and R. Jenness, University of Minnesota.

M53. Kappa-casein–β-lactoglobulin interaction in solution when heated. C. A. Zittle, Eastern Utilization Research and Development Division, USDA, Philadelphia.

8:30–10:45 A.M.

Section C. **Dairy Manufacturing Extension**

W. S. Arbuckle, Chairman

Moore Hall, Room 102

Modern concepts of dairy plant management. C. E. French, Purdue University.

Applying results of research to industry. V. H. Nielsen, Iowa State University.

Profitable application of automation for handling products and cleaning-in-place. R. P. March, Cornell University.

Dairy Manufacturing Extension Business Meeting

11:00–12:00 M.

Manufacturing Section Business Meeting.

B. Heinemann, Chairman

Babcock Hall, Room 205

1:30– 4:30 P.M.

Section A. **Dairy Chemistry**

D. M. Graham, Chairman

Babcock Hall, Room 205

M54. Some carbonyl compounds formed by the action of sunlight on milk. L. A. Wishner and M. Keeney, University of Maryland.

M55. Mass spectrometry and gas chromatography applied to the study of volatiles in milk. R. Bassette and C. H. Whitnah, Kansas State University.

M56. Hydrolysis of certain organo-phosphorus enzyme inhibitors by raw milk. T. L. Forster, Washington State University.

M57. Observations on the purification of B-esterase from bovine milk by adsorption on $Mg(OH)_2$. M. W. Montgomery and T. L. Forster, Washington Agricultural Experiment Station.

M58. An ultrasonic method for measurement of solids-not-fat and milk fat in fluid milk. I. Acoustic properties. J. W. Fitzgerald, Chesapeake Instrument Corporation, Shadyside, Maryland, G. R. Ringo, Argonne National Laboratories, La Grange Park, Illinois, and W. C. Winder, University of Wisconsin.

M59. An ultrasonic method for measurement of solids-not-fat and milk fat in fluid milk. II. An evaluation of the method. W. C. Winder, N. P. Consigny, and B. Rodriguez-Lopez, University of Wisconsin.

M60. Determination of density of milk by gamma ray attenuation. B. J. Demott and H. C. Holt. UT—AEC Agricultural Research Laboratory, Oak Ridge and University of Tennessee.

M61. A study of the TBA reaction as a measure of oxidized flavor in milk. R. L. King, University of Maryland.

M62. Factors affecting the pH-sensitivity of the heat stability of milk from individual cows. Dyson Rose, Division of Applied Biology, National Research Council, Ottawa, Canada.

M63. A method for the removal of cationic radionuclides from milk. G. K. Murthy, E. B. Masurovsky, L. F. Edmondson, and J. E. Campbell, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, and USDA, Beltsville, Maryland.

M64. The removal of in vivo and in vitro labeled radio-strontium from milk and its distribution between centrifugally separated phases. L. F. Edmondson, D. H. Keefer, F. W. Douglas, Jr., J. Y. Harris, and E. Dodson, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

M65. Optimum ratio of cations on charged resin for removal of radio-strontium from milk. A. R. Landgrebe, L. F. Edmondson, and F. W. Douglas, Jr., Eastern Utilization Research and Development Division, USDA, Washington, D. C.

1:30–4:30 P.M.

Section B. **Cheese and Butter**
J. E. Edmondson, Chairman
Biochemistry, Room 101

M66. The crystallization rate of milk fat. J. M. de Man, University of Alberta, Edmonton, Canada.

M67. Conditions affecting the rearrangement of milk fat. J. B. Mickle, R. L. Von Gunten, and R. D. Morrison, Oklahoma State University.

M68. Studies on the thermodynamics of butter and its physical state. J W. Sherbon and S. T. Coulter, University of Minnesota.

M69. Dilatometric behavior and other properties of modified milk fats. R. R. Riel, Dairy Technology Research Institute, Ottawa, Canada.

M70. The effect of homogenization on the hardness, oiling-off percentages, and water dispersion of butter. F. W. Wood and L. M. McKnight, University of Alberta, Edmonton, Canada.

M71. Blue mold cheese as a potential agent of chronic penicillin urticaria. R. A. Ledford and F. V. Kosikowski, Cornell University.

M72. The effect of varying age blends of Cheddar cheese on the stability of Cheddar cheese spreads for the Armed Forces. R. I. Meyer, D. F. Wilcox, C. C. Walts, and G. M. Steed, Quartermaster Food and Container Institute for the Armed Forces, Quartermaster Research and Engineering Command, U. S. Army, Chicago.

M73. The B-complex vitamins content of cheese. K. M. Shahani, I. L. Hathaway, and P. L. Kelly, University of Nebraska.

M74. A method for the analysis of cheese volatiles. W. A. McGugen and S. G. Howsam, Canada Department of Agriculture, Ottawa, Canada.

1:30–4:30 P.M.

Section C. **Dairy Manufacturing Extension**
R. B. Redfern, Chairman
Moore Hall, Room 102

Handling the world's dairy literature. E. J. Mann, Director, Commonwealth Bureau of Dairy Science and Technology, Shinfield, Reading, Berks, England.

New products and processes in the dairy industry. K. G. Weckel, University of Wisconsin.

The "firsts" we used in our Milwaukee plant. W. E. Shiffermiller, The Borden Company, Milwaukee, Wisconsin.

How the dairy industry can profitably use the services of the dairy technology specialist.

The College's Viewpoint	C. B. Ratchford University of Missouri
Industry's Viewpoint	Hubert Garrecht Klinke Bros. Dairy, Memphis

4:30–5:30 P.M.

Informal Meeting for Discussion of Cheese Cultures.

R. Tittsler, Chairman
Babcock Hall, Room 121

Wednesday, June 14

8:00–9:45 A.M.

All Sections

Symposium: Protein Methodology

C. A. Ernstrom, Chairman
Babcock Hall, Room 205

Chromatography of milk proteins on D.E.A.E. cellulose. N. P. Tarassuk, University of California.

Small angle X-ray scattering in protein studies. Paul Schmidt, Rice Institute.

Physical chemical studies of the association of the genetic species of beta-lactoglobulin. S. N. Timarsheff, Eastern Utilization Research and Development Division, USDA, Philadelphia.

10:00–11:00 A.M.

Joint with Production and Extension Sections

Invitational paper: Field Problems with Pesticide Residues.

H. E. O. Heinemann, Pet Milk Company

Discussant: J. E. Casida, Department of Entomology, University of Wisconsin
Agricultural Hall Auditorium

11:00 A.M.–12:30 P.M.

General Session Devoted to Association Affairs

Agricultural Hall Auditorium

1:30–3:30 P.M.

Section A. **Milk Lipids**

B. Heinemann, Chairman
Babcock Hall, Room 205

M75. Comparative hydrolysis of primary and secondary ester positions of glycerol by milk lipases. G. W. Gander, R. G. Jensen, and J. Sampugna, Agricultural Experiment Station, Storrs, Connecticut.

M76. Fatty acid composition of the diglycerides from lipolyzed milk fat. R. G. Jensen, G. G. Gander, and J. Sampugna, Agricultural Experimental Station, Storrs, Connecticut.

M77. Specificity of milk lipases. V. R. Harwalkar and H. E. Calbert, University of Wisconsin.

M78. Comparison of fatty acid composition of milk fats produced by cows fed alfalfa, oat, or ground pelleted alfalfa hay. L. M. Smith and M. Ronning, University of California.

M79. The relation of linoleate and linolenate to the flavor of autoxidized butteroil. E. G. Hammond, A. M. El Negoumy, and M. S. de Puchal, Iowa State University.

M80. Characteristics of the fat-globule membrane lipoproteins. J. R. Brunner and M. P. Thompson, Michigan State University.

M81. A method for determining the free fatty acids in milk with gas chromatography. J. A. Trammel and J. J. Janzen, Clemson College, Clemson.

M82. Heat-induced changes in milk fat. W. W. Nawar and I. S. Fagerson, University of Massachusetts.

1:30–3:30 P.M.

Section B. **Cottage Cheese and Ice Cream**

M. L. Speck, Chairman

Biochemistry, Room 101

M83. Spray drying Cottage cheese whey. F. P. Hanrahan and B. H. Webb, Eastern Utilization Research and Development Division, USDA, Washington, D. C.

M84. The effect of increasing the serum protein content of Cottage cheese curd on yield and quality. N. W. Durrant, W. K. Stone, and P. M. Large, Virginia Polytechnic Institute.

M85. The effect of potassium sorbate on organisms commonly associated with Cottage cheese spoilage. R. L. Bradley, Jr., and L. G. Harmon, Michigan State University.

M86. Hardening of ice cream products with vertical refrigerated plates. J. C. Barnes, T. I. Hedrick, and C. M. Stine, Michigan State University.

M87. The effect of ultra high temperatures on certain properties of ice cream. E. S. Humbert and D. L. Gibson, University of Saskatchewan, Saskatoon, Canada.

M88. Identification of flavoring components of chocolate products for ice cream manufacturing. K. S. Ramachandran and I. A. Gould, Ohio State University.

M89. Organoleptic evaluation of chocolate flavoring materials for ice cream. K. S. Ramachandran and I. A. Gould, Ohio State University.

M90. Consumer preferences for vanilla ice cream with different amounts of sugar. E. J. Finnegan and J. J. Sheuring, University of Georgia.

PRODUCTION SECTION

Monday, June 12

9:00–10:30 A.M.

Opening Session
Wisconsin Union Theater

10:30–11:45 A.M.

Program Sponsored by Education Committee
Wisconsin Union Theater

1:30–4:30 P.M.

Section A. **Feed Utilization**
J. C. Thompson, Chairman
Commerce Auditorium

P1. Reliability of score card judging of hay. T. P. Fairchild, E. L. Corley, and G. M. Werner, University of Wisconsin.

P2. Effect of expanded concentrates, ground concentrates, and completely pelleted rations on ration digestibility and dairy heifer growth. D. W. Burton, G. F. W. Haenlein, and C. R. Richards, University of Delaware.

P3. Digestion in the steer, goat, and artificial rumen as measures of the nutritive value of alfalfa harvested at four stages of maturity. W. J. Byer, H. F. Jumah, B. R. Baumgardt, and R. P. Niedermeier, University of Wisconsin.

P4. The effect of the physical form on the nutritive value of hay fed to lactating dairy cows. J. M. Keith, W. A. Hardison, J. T. Huber, and G. C. Graf, Virginia Agricultural Experiment Station.

P5. Apparent digestibility for the dry matter, crude protein, and energy of alfalfa with advancing maturity. F. A. Martz, C. H. Noller, and D. L. Hill, Purdue University.

P6. Nutrient value of forages. I. Yield and digestibility of nutrients at two stages of development. J. H. Byers and E. E. Ormiston, University of Illinois.

P7. Digestibility of timothy forage as affected by date of harvest. T. N. Mellin, B. R. Poulton, and M. J. Anderson, University of Maine.

P8. Effect of low, medium, and high fiber supplements on the performance of lactating cows on pasture. F. G. Owen and G. E. Van Riper, University of Nebraska.

P9. Effect on milk production, body weight, and dry matter consumption when feeding haylage, hay, and silage, with simple concentrate supplements. H. J. Larsen and R. F. Johannes, University of Wisconsin.

P10. Digestibility and intake of corn, oats, and sorghum silages. D. C. Foss, R. P. Niedermeier, B. R. Baumgardt, and R. D. Lance, University of Wisconsin.

P11. Nutritive value of Ambercane silage for milking cows, sheep, and rabbits. G. F. W. Haenlein and C. R. Richards, University of Delaware.

P12. Chemical quality and feeding value of orchardgrass silage as affected by nitrogen fertilization. J. C. Derbyshire, C. H. Gordon, H. G. Wiseman, C. G. Melin, and E. A. Kane, Animal Husbandry Research Division, USDA, Beltsville, Maryland.

P13. Thyroprotein feeding on a one hundred–cow commercial dairy farm. E. W. Colette, T. M. Sutherland, R. G. Hinders, and G. M. Ward, Colorado State University.

P14. Whole cottonseed in limited-fiber rations for dairy cattle. E. G. Moody and A. P. Cook, Arizona State University.

1:30–4:30 P.M.

Section B. **Nutrition**

L. H. Schultz, Chairman

Commerce, Room 22

P15. Urea in dairy ration. V. K. Singh and J. D. Donker, University of Minnesota.

P16. Factors affecting the excretion pattern of chromic oxide in feces of dairy cows on pasture. A. C. Linnerud and J. D. Donker, University of Minnesota.

P17. Estimated net energy and TDN relationships of classes of feeds. E. A. Kane, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

P18. The use of detergents in the analysis of forages and determination of the effects of heat-drying upon proteins. P. J. Van Soest, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

P19. A comparative study of freezing, canning, and drying as methods of preserving bovine excreta for chemical analysis. W. P. Flatt, H. F. Righter, D. R. Gilliam, and H. F. Lawson, Animal Husbandry Research Division, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

P20. Is hay required to develop rumen capacity? R. G. Warner, Cornell University.

P21. Effects of feeding dehydrated alfalfa pellets as the only roughage to dairy cows. R. G. Hinders, Gy. Vidacs, and G. M. Ward, Colorado State University.

P22. A quantitative in vivo study of rumen capacity as influenced by milk and dry feed consumption. H. N. Harrison and R. G. Warner, Cornell University.

P23. The use of the rat to study changes of rumen epithelium (parakeratosis) occurring on dehydrated alfalfa pellet diet. Gy. Vidacs, R. G. Hinders, and G. M. Ward, Colorado State University.

P24. Multiple regression analysis of responses to dietary calcium, phosphorus, and vitamin D_3 in young rats. P. T. Chandler and R. G. Cragle, University of Tennessee—Atomic Energy Commission Agricultural Research Laboratory, Oak Ridge, and University of Tennessee.

P25. The effect of feeding sodium and potassium bicarbonate on milk fat, rumen pH, and organic acid production. R. S. Emery and L. D. Brown, Michigan State University.

P26. A comparison of various methods for the determination of acetic acid in venous blood of the bovine. C. L. Davis and R. E. Brown, University of Illinois.

P27. Further studies on a salivation factor (SF) in leguminous forages. J. H. Byers and H. P. Broquist, University of Illinois.

P28. Acetate metabolism in fed sheep. J. R. Sabine and B. Connor Johnson, University of Illinois.

1:30–4:30 P.M.

Section C. **Artificial Breeding**
S. D. Musgrave, Chairman
Commerce, Room 19

P29. Elimination of *Vibrio fetus* from bovine sperm stored at low temperatures. C. Norman, J. L. McCoy, I. D. Porterfield, and H. A. Wilson, West Virginia University.

P30. Prolonged maintenance of bovine sperm in a chemically defined diluent at ambient temperatures (21-27° C.). C. Norman, C. E. Johnson, I. D. Porterfield, E. Goldberg, and R. S. Dunbar, Jr., West Virginia University.

P31. Effect of catalase in semen extenders on fertility, and on sperm survival at high extension rates. R. H. Foote, Cornell University.

P32. Transport of galactose by bovine spermatozoa. R. J. Flipse, Pennsylvania State University.

P33. The effect of nicotinamide on the livability of bovine spermatozoa during storage. E. E. Jones and G. W. Salisbury, University of Illinois.

P34. The metabolism of intermediary metabolites by mammalian spermatozoa. C. N. Graves, University of Illinois.

P35. Anaerobic metabolism of epididymal-like bovine spermatozoa and a requirement for carbon dioxide. J. R. Lodge, University of Illinois.

P36. Chemical analysis of deoxyribonucleic acid (DNA) of bovine spermatozoa. G. W. Salisbury and J. R. Lodge, University of Illinois.

P37. Spermatozoa-specific antigens in the bovine. M. J. Gordon and A. G. Hunter, Michigan State University.

P38. Direct and indirect measurements of testicular daily sperm production (DSP). R. P. Amann and J. O. Almquist, Pennsylvania State University.

P39. The effect of frequency of ejaculation on the freezing and storage of bovine spermatozoa in two extenders. B. S. Ahluwalia, G. R. Fisher, W. E. Erickson, and E. F. Graham, University of Minnesota.

P40. An investigation of the source or cause of coiled taills on bovine spermatozoa. E. W. Swanson and L. J. Boyd, University of Tennessee.

P41. Effect of storage temperature of bovine spermatozoa on motility and fertility. G. R. Fisher and E. F. Graham, University of Minnesota.

P42. Enzymatic degradation of hydrogen peroxide in egg yolk. C. Desjardins and H. D. Hafs, Michigan State University.

Tuesday, June 13

8:30–10:45 A.M.

Section A. **Calf Nutrition**

J. C. Thompson, Chairman
Commerce, Room 19

P43. The effect of dietary dilution on nutrient intake by dairy calves. J. D. Pettyjohn, J. P. Everett, Jr., and R. D. Mochrie, North Carolina State College.

P44. The effect of diet and of age on the rate of absorption of acetic acid from the reticulo-rumen of the young calf. J. D. Sutton, A. D. McGilliard, and N. L. Jacobson, Iowa State University.

P45. The response of experimentally produced zinc-deficient calves to dietary zinc. J. K. Miller and W. J. Miller, University of Georgia.

P46. Alterations in body hydration of growing calves as measured by thiocyanate space. T. R. Wrenn, J. Bitman, and M. Okamoto, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

P47. Further studies on anemia in newborn dairy calves. J. W. Hibbs, H. R. Conrad, and C. Gale, Ohio Agricultural Experiment Station.

P48. Effects of level of ration intake and duration of vitamin A deficiency upon some biochemical constituents in serum, cerebrospinal fluid, and aqueous humor of Holstein calves fed fixed carotene intakes. D. G. Hazzard, A. P. Grifo, Jr., J. E. Rousseau, Jr., C. G. Woelfel, H. D. Eaton, S. W. Nielsen, and D. G. Gosslee, Storrs (Connecticut) Agricultural Experiment Station.

P49. Protein-energy ratio studies in dairy calves. L. D. Brown and C. A. Lassiter, Michigan State University.

P50. A nitrofuran in whole milk and in a milk replacer. A. S. Wood, J. J. Jezeski, and J. B. Williams, University of Minnesota.

P51. A comparison of the anthrone reaction with a copper-reduction method for the estimation of the blood sugar of calves. H. W. Colvin, Jr., J. T. Attebery, and J. T. Ivy, University of Arkansas.

P52. Electrophoretic separation of esterases present in various tissues of the calf. H. A. Ramsey, North Carolina State College.

8:30–10:45 A.M.

Section B. **Reproductive Physiology**
L. H. Schultz, Chairman
Commerce, Room 22

P53. The effect of repeated intrauterine inoculations with bacteria on subsequent fertility in heifers. A. C. Menge, A. J. Winter, S. H. McNutt, and L. E. Casida, University of Wisconsin.

P54. The influence of exogenous progesterone upon post-partum reproductive activities in the bovine. O. T. Fosgate, University of Georgia.

P55. Effect of progesterone on breeding efficiency in dairy cattle subjected to high environmental temperature. G. H. Stott and R. J. Williams, University of Arizona.

P56. The formation of the bovine corpus luteum. H. T. Gier and G. B. Marion, Kansas State University.

P57. Luteal function and embryo survival in the bovine. R. E. Staples and W. Hansel, Cornell University.

P58. Luteal function as related to embryo development and pituitary and ovarian cytology in the bovine. R. E. Staples, W. Hansel, and K. McEntee, Cornell University.

P59. Gross differentiation of the bovine uterus. E. P. Call and G. B. Marion, Kansas State University.

P60. Effect of feeding 10 mg. stilbestrol daily on growth and breeding efficiency of dairy heifers. G. R. Krawczyk and H. H. Olson, Southern Illinois University.

P61. Metabolic activity of rabbit testicular tissue after various periods in the abdomen. L. L. Ewing and N. L. VanDemark, University of Illinois.

P62. Descent of the testis in the bovine. E. B. Krehbiel and H. T. Gier, Kansas State University.

8:30–10:45 A.M.

Section C. **Genetics**

S. D. Musgrave, Chairman

Commerce Auditorium

P63. Effectiveness of statistical adjustments for yearly fluctuations in production. R. F. Gaalaas and R. D. Plowman, Dairy Cattle Research Branch, Animal Husbandry Research Division, USDA, Beltsville, Maryland.

P64. Accuracy of evaluating A.I. sires in selected herds. H. W. Carter, Cornell University.

P65. The heritability of milk yield at different levels of production and the effect of production differences on dairy sire appraisals. E. B. Burnside and J. C. Rennie, Ontario Agricultural College.

P66. Effects of herd, breed, sire, and season on persistency of milk production. O. T. Stallcup, J. M. Rakes, and O. H. Horton, University of Arkansas.

P67. Caesarean sections as a genetic and embryological research tool. Keith Huston, J. L. Noordsy, S. T. Smith, E. P. Call, and F. H. Oberst, Kansas State University.

P68. Relationships between solids-not-fat and other constituents and properties of milk. W. A. Krienke and C. J. Wilcox, Florida Agricultural Experiment Station.

P69. Relative protein content of milk produced by four breed groups in a tropical environment. J. V. Bateman and J. de Alba, Inter-American Institute of Agricultural Sciences, Costa Rica.

P70. Vital statistics of Holstein dairy cattle and their effect on herd increase. A. M. Meak and J. L. Lush, Iowa State University.

P71. Some genetic and environmental aspects of certain performance traits in a Louisiana Holstein herd. C. Branton, D. L. Evans, and W. H. Waters, Louisiana State University.

P72. Environmental factors affecting nonfat-solids and protein content of milk. J. M. Treece, L. O. Gilmore, N. S. Fechheimer, and R. G. Washburn, Ohio Agricultural Experiment Station.

11:00–12:00 M.

Production Section Business Meeting
S. D. Musgrave, Chairman
Commerce Auditorium

1:30–4:30 P.M.

Section A. **Joint with Extension Section**

Symposium: A Modern Dairy Cattle Feeding Program
George Werner, Chairman
Agricultural Hall Auditorium

Results of feed analysis in feeding dairy cattle. R. S. Adams, Pennsylvania State University.

High level grain feeding for dairy cows. C. F. Huffman, Michigan State University.

Problems of feed evaluation related to the feeding of dairy cows. J. T. Reid, Cornell University.

Using feed information on the farm. G. H. Porter, Beacon Milling Company.

1:30–4:30 P.M.

Section B. **Physiology**
L. H. Schultz, Chairman
Commerce, Room 19

P73. Effect of sudden exposure to hot conditions on physiological functions of dairy heifers. J. E. Johnston, G. A. Hindery, D. H. Hill, and A. J. Guidry, Louisiana Agricultural Experiment Station.

P74. Role of heat tolerance and production level in responses of lactating Holsteins to various temperature humidity conditions. H. D. Johnson, H. H. Kibler, A. C. Ragsdale, University of Missouri; I. L. Berry and M. D. Shanklin, Agricultural Engineering Research Division, USDA, Beltsville, Maryland.

P75. Vaporization rates of Brown Swiss, Holstein, and Jersey calves at air temperatures ranging from 33 to 95° F. R. G. Yeck and I. L. Berry, Agricultural Engineering Research Division, USDA, Beltsville, Maryland; H. H. Kibler, University of Missouri.

P76. Fallout levels of cesium^{137} in milk in the U. S. as influenced by feeding practices. G. M. Ward, Los Alamos Scientific Laboratory, University of California.

P77. Hydrocortisone secretion rate in the cow. R. R. Anderson, G. W. Pipes, W. R. Miller, and C. W. Turner, University of Missouri.

P78. Mammary gland growth in mice. R. R. Anderson and C. W. Turner, University of Missouri.

P79. Growth of mammary glands of suckling ovariectomized hooded Norway rats injected with ACTH and estrogen. G. J. Macdonald and R. P. Reece, New Jersey Agricultural Experiment Station.

P80. Isolation and preliminary characterization of a gastrin-like gastric secretory stimulant from pyloric mucosa of the bovine abomasum. W. R. Anderson, T. L. Fletcher, C. L. Pitts, R. L. Cohen, and H. N. Harkins, Department of Surgery, University of Washington, Seattle.

P81. Effects of parathyroid extract (PTE) on calcium, phosphorus, magnesium, and citric acid metabolism in dairy cattle. A. S. Todd, O. T. Fosgate, R. G. Cragle, and T. H. Kamal, University of Tennessee—Atomic Energy Commission Agricultural Research Laboratory, Oak Ridge, and University of Georgia.

P82. The influence of lactose and sucrose on calcium balances of mature dairy cows. S. M. Badawi and T. H. Blosser, Washington State University.

P83. Portal blood studies in the bovine. D. E. Waldern, T. H. Blosser, O. L. Frost, and J. A. Harsch, Washington State University.

P84. The distribution of thiocyanate between cerebrospinal fluid and blood in control and vitamin A–deficient calves. M. Okamoto, J. Bitman, and T. R. Wrenn, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

P85. Thyroxine and secretion of I^{131} in milk in cattle. B. N. Premachandra and C. W. Turner, University of Missouri.

P86. The effects of breed, stage of lactation, and season of year on thyroid secretion rate in dairy cows as determined by the chemical thyroxine turnover method. J. P. Mixner, New Jersey Agricultural Experiment Station.

1:30–4:30 P.M.

Section C. **Reproductive Physiology and Genetics**
S. D. Musgrave, Chairman
Commerce Auditorium

P87. Reproductive function in male goats in various thyroid states. J. A. Williams and E. P. Reineke, Michigan State University.

P88. The control of estrus and ovulation in heifers by orally administered 6-α-methyl-17-α-acetoxyprogesterone. R. G. Zimbleman, The Upjohn Company, Kalamazoo, Michigan.

P89. Synchronization of estrus in heifers with 6-α-methyl-17-α-acetoxyprogesterone and its effect on subsequent ovulation and fertility. W. E. Collins, L. W. Smith, E. R. Hauser, and L. E. Casida, University of Wisconsin.

P90. Effect of ovarian hormones and uterine infection on capillary permeation in the rabbit endometrium. H. W. Hawk, G. D. Turner, and T. H.

Brinsfield, Animal Husbandry Research Division, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

P91. Interrelationships among some factors affecting post-natal development of dairy calves. E. J. Stone, B. R. Farthing, and C. Branton, Louisiana State University.

P92. Some factors affecting milk fat and protein contents in milk from dairy herds in Central Texas. M. A. Brown, C. Vanderzant, B. A. Parker, and I. W. Rupel, A & M College of Texas.

P93. Some findings of a survey of 50 Virginia dairy herds relative to milk solids-not-fat. A. C. Holliday, Virginia Department of Agriculture; N. R. Thompson, Virginia Polytechnic Institute.

P94. Effect of month of calving in herds at three levels of production. B. Bereskin and A. E. Freeman, Iowa State University.

P95. Some hereditary and environmental factors associated with congenital abnormalities in cattle. M. S. Herschler, N. S. Fechheimer, and L. O. Gilmore, Ohio State University.

P96. Body measurements and their use in predicting milk production. A. E. Freeman and K. L. Roache, Iowa State University.

P97. Relation of days open and days dry to lactation production. J. W. Smith and J. E. Legates, North Carolina State College.

P98. Genetics of syndactylism in cattle. Keith Huston, F. E. Eldridge, and J. W. Mudge, Kansas State University.

P99. Combinations of internal hydrocephalus, congenital edema, and extra ear lobes in Ayrshire cattle. W. H. Cloninger, J. R. Nichols, and J. F. Hokanson, Pennsylvania State University.

4:30–5:30 P.M.
Joint with Extension Section

Joint Committee Reports
M. E. Senger, Chairman

Agricultural Hall Auditorium
Dairy Cattle Breeding. R. E. Corbett, Maine
Dairy Cattle Health. V. R. Smith, Arizona
Dairy Cattle Type. R. Wayne, Minnesota

Wednesday, June 14

8:00–9:45 A.M.
Section A. **Calf Nutrition**
J. C. Thompson, Chairman
Commerce, Room 19

P100. Enzyme supplements in calf starters. J. W. Rust, N. L. Jacobson, A. D. McGilliard, and C. F. Foreman, Iowa State University.

P101. Studies to define the secretory site of pre-gastric esterase in calves. J. H. Nelson, Dairyland Food Laboratories, Inc., Waukesha, Wisconsin; W. B. House and Vere English, Midwest Research Institute, Kansas City, Missouri; and T. Y. Lou, Kansas University Medical Center, Kansas City, Kansas.

P102. The treatment of calf scours with pre-gastric esterase (PGE) preparations. J. H. Nelson, Dairyland Food Laboratories, Inc., Waukesha, Wisconsin; W. B. House and Vere English, Midwest Research Institute, Kansas City, Missouri.

P103. Effect of spiramycin on the growth of young dairy calves. C. A. Lassiter, L. D. Brown, and R. O. Thomas, Michigan State University.

P104. The response of young dairy calves to diets containing sesame and peanut oils. Dayal Dass Shrivastava and K. A. Kendall, University of Illinois.

P105. Incorporating coarse ground hay into the starter ration of early-weaned calves. C. H. Noller, D. L. Hill, F. A. Martz, and B. W. Crowl, Purdue University.

P106. The influence of stilbestrol with a high roughage ration in raising replacement dairy heifers. Myron G. A. Rumery and G. M. Ward, Colorado State University.

8:00–9:45 A.M.

Section B. **Joint with Extension Section**

Symposium: Recent Advances in Reproductive Efficiency

S. D. Musgrave, Chairman

Commerce Auditorium

Estrous cycle and ovulation control. W. Hansel, Cornell University.

Artificial insemination. N. L. VanDemark, University of Illinois.

The present status of the repeat breeding problem. L. E. Casida, University of Wisconsin.

10:00–11:00 A.M.

Joint with Extension and Manufacturing Sections

Invitational Paper: Field Problems with Pesticide Residues

H. E. O. Heinemann, Pet Milk Company.

Discussant: J. E. Casida, Department of Entomology, University of Wisconsin.

Agricultural Hall Auditorium

11:00 A.M.–12:30 P.M.

General Session Devoted to Association Affairs

Agricultural Hall Auditorium

1:30–3:30 P.M.

Section A. **Milk Production**

S. D. Musgrave, Chairman

Commerce Auditorium

P107. Hand versus machine milking. N. W. Hooven, Jr. and R. D. Plowman, Dairy Cattle Research Branch, Animal Husbandry Research Division, USDA, Beltsville, Maryland.

P108. Lactational response of dairy cows injected or implanted with growth hormone. D. D. Bullis, B. Harris, Jr., and L. J. Bush, Oklahoma State University.

P109. Incidence of antibiotics in milk following intramuscular treatment. G. R. Krawczyk and H. H. Olson, Southern Illinois University.

P110. Synthesis of β-lactoglobulin in bovine mammary cell cultures. B. L. Larson and Judith Twarog, University of Illinois.

P111. The effect of prepartum intramammary infusions on milk production. H. Struss, W. E. Petersen, and B. Campbell, University of Minnesota.

P112. The effect of postpartum intramammary infusion of a bacterial antigen on milking Guernsey cows. H. Struss, W. E. Petersen, and B. Campbell, University of Minnesota.

P113. Labor requirements for three systems of milking in a stanchion barn. R. D. Plowman and N. W. Hooven, Jr., Dairy Cattle Research Branch, Animal Husbandry Research Division; D. R. Mackay, Farm Electrification Research Branch, Agricultural Engineering Research Division, USDA, Beltsville, Maryland.

P114. An attempt to measure experimentally the possible mastitogenic effect of several milking machine factors. J. M. Murphy, University of Pennsylvania.

P115. Residues in milk fat from dairy cows grazing pastures treated with granular heptachlor for eradication of the imported fire ant. L. L. Rusoff, W. H. Waters, J. H. Gholson, and J. B. Frye, Jr., Dairy Department; L. D. Newsome and E. C. Burns, Entomology Department, Louisiana Agricultural Experiment Station.

1:30–3:30 P.M.

Section B. **Rumen Physiology**

L. H. Schultz, Chairman

Commerce, Room 22

P116. Effect of animal mucin concentrates on bloat. E. E. Bartley and I. S. Yadava, Kansas State University.

P117. Role of mucinolytic bacteria in feedlot bloat. L. R. Fina, E. E. Bartley, and B. Mishra, Kansas State University.

P118. The relation of pentosans and polyuronides in forage plants to the incidence of pasture bloat. H. R. Conrad and B. A. Dehority, Ohio Agricultural Experiment Station.

P119. An apparatus for the continuous cultivation of the rumen microbial population. W. H. Rufener, W. O. Nelson, and M. J. Wolin, University of Illinois.

P120. Effect of diet on lactate-C^{14} fermentation in rumen fluid. R. L. Baldwin, W. A. Wood, R. S. Emery, and M. Wacasey, Michigan State University.

P121. Effect of ad libitum or equalized feeding of alfalfa hay or silage on rumen contents and its characteristics. J. W. Thomas, J. R. Ingalls, M. Yang, and B. S. Reddy, Michigan State University.

P122. Production of volatile fatty acids and lactic acid from corn starch by rumen microorganisms in vitro. R. L. Salsbury, J. A. Hoefer, and R. W. Luecke, Michigan State University.

P123. The metabolic fate of isovalerate in *Ruminococcus flavefacien*. M. J. Allison and M. P. Bryant, Dairy Cattle Research Branch, USDA, Beltsville, Maryland, and M. Keeney and I. Katz, University of Maryland.

P124. Ruminal absorption of amino acids. R. M. Cook, R. E. Brown, and C. L. Davis, University of Illinois.

P125. Proportions of volatile fatty acids in rumen ingesta of dairy heifers following changes from one type of roughage to another. R. E. Roffler and F. R. Murdock, Washington State University.

P126. The effect of lactate feeding on ketosis in dairy cattle. H. D. Radloff and L. H. Schultz, University of Wisconsin.

1:30–3:30 P.M.

Section C. **Forages**

J. C. Thompson, Chairman

Commerce, Room 19

P127. Effectiveness of enzyme and culture additions on the preservation and feeding value of alfalfa silage. M. Olson and H. H. Voelker, South Dakota State College.

P128. The effect of zinc bacitracin on silage microorganisms. C. W. Langston, R. M. Conner, C. H. Gordon, and L. A. Moore, Dairy Cattle Research Branch, USDA, Beltsville, Maryland.

P129. The effects of stage of maturity, wilting, and a preservative on losses in the silo. Hagen Lippke and R. E. Leighton, A & M College of Texas.

P130. Zinc bacitracin as a silage preservative. A. D. Pratt and H. R. Conrad, Ohio Agricultural Experiment Station.

P131. Comparative feeding value of corn and grain sorghum silages. C. B. Browning, J. W. Lusk, and J. T. Miles, Mississippi State University.

P132. Effect of soybean flakes and rate of filling on nutrient losses and silage quality. W. J. Miller, C. M. Clifton, and N. W. Cameron, University of Georgia.

P133. Organic solvents for forage preservation. L. D. Brown and R. S. Emery, Michigan State University.

P134. The effect of preservatives on silage dry matter losses in sweet and grain sorghums. D. S. Ramsey, Mississippi State University.

P135. The digestibility of common Timothy hays grown with different levels of nitrogen fertilization. R. E. Stoker, J. L. Evans, M. C. Stillions, W. V. Chalupa, and C. H. Ramage, Rutgers University.

EXTENSION SECTION

Monday, June 12

9:00–11:45 A.M.

Opening Session

Wisconsin Union Theater

10:30–11:45 A.M.

Program Sponsored by Education Committee

Wisconsin Union Theater

1:30–4:30 P.M.

Teaching Methods

James W. Crowley, Chairman
Bacteriology Auditorium

E1. An extension program in a changing agriculture. H. L. Ahlgren, Associate Director Cooperative Extension, University of Wisconsin.

Panel Discussion: The Team Approach to Problem Solving.

Moderator: R. E. Burleson, Federal Extension Service, USDA.

E2. Working with related specialists. C. D. McGrew, Ohio State University.

E3. How related specialists view the team approach. F. V. Burcalow, Department of Agronomy, University of Wisconsin.

E4. Administrative assistance in attacking the problem. J. R. Parrish, Auburn University.

E5. Extension and industry working together. M. F. Ellmore, Virginia Polytechnic Institute.

Presentation and explanation of exhibits.

4:30–5:30 P.M.

Joint with Production Section

Meeting of Superintendents of Official Testing

W. R. Van Sant, Chairman

Bacteriology Auditorium

Tuesday, June 13

8:30–10:15 A.M.

4-H Club Program

J. E. Cooper, Chairman

Bacteriology Auditorium

E6. 4-H Calf Club project can help provide herd replacements. F. N. Knott, North Carolina State College.

E7. Dairy 4-H Club work for the sixties. M. S. Downey, Director 4-H Club Division, USDA.

10:30–12:00 M.

Extension Section Business Meeting

M. E. Senger, Chairman

Bacteriology Auditorium

Report of Teaching Methods Committee

J. W. Crowley, Chairman

Report of 4-H Club Committee

J. E. Cooper, Chairman

Report of Resolutions Committee

Fred Meinershagen, Chairman

Report of Dairy Records Committee

W. R. Van Sant, Chairman

Report of the Nominating Committee

1:30–4:30 P.M.

Joint with Production Section

Symposium: A Modern Dairy Cattle Feeding Program

George Werner, Chairman

Agricultural Hall Auditorium

E8. Results of feed analysis in feeding dairy cattle. R. S. Adams, Pennsylvania State University.

E9. High level grain feeding for dairy cows. C. F. Huffman, Michigan State University.

E10. Problems of feed evaluation related to the feeding of dairy cows. J. T. Reid, Cornell University.

E11. Using feed information on the farm. G. H. Porter, Beacon Milling Company.

4:30–5:30 P.M.
Joint with Production Section

Joint Committee Reports
M. E. Senger, Chairman
Agricultural Hall Auditorium

Dairy Cattle Breeding. R. E. Corbett, Maine
Dairy Cattle Health. V. R. Smith, Arizona
Dairy Cattle Type. R. Wayne, Minnesota

Wednesday, June 14

8:00–9:45 A.M.
Joint with Production Section

Symposium: Recent Advances in Reproductive Efficiency
S. D. Musgrave, Chairman
Commerce Auditorium

Estrous cycle and ovulation control. W. Hansel, Cornell University.
Artificial insemination. N. L. VanDemark. University of Illinois.
The present status of the repeat breeding problem. L. E. Casida, University of Wisconsin.

10:00–11:00 A.M.
Joint with Manufacturing and Production Sections.

Invitational Paper: Field Problems with Pesticide Residues
H. E. O. Heinemann, Pet Milk Company

Discussant: J. E. Casida, Department of Entomology, University of Wisconsin
Agricultural Hall Auditorium

11:00 A.M.–12:30 P.M.
General Session Devoted to Association Affairs.
Agricultural Hall Auditorium

1:30–4:30 P.M.
Dairy Records and the New Proved Sire Report
W. R. Van Sant, Chairman
Bacteriology Auditorium
An Action Program to Improve DHIA Records.

E12. A training program for DHIA supervisors and county agents. R. C. Fincham, Iowa State University.

E13. Checking DHIA supervisors in their testing and record-keeping work. C. H. Parsons and D. A. Evans, University of Massachusetts.

E14. Enforcing the rules and regulations of the DHIA program. H. C. Gilmore, Pennsylvania State University.

E15. Handling DHIA problems in the electronic processing center. G. V. O'Bleness, Cornell University.

Panel Discussion: Problems of Dairy Cattle Identification.
Moderator: E. C. Meyer, *Hoard's Dairyman.*

E16. The importance of proper identification to the DHIA supervisor. K. Finch, Winnebago County, Wisconsin.

E17. The importance of proper identification to the breed associations. C. H. Bohl, American Jersey Cattle Club.

E18. The procedure and methods used in identifying cows in large herds. C. L. Pelissier, University of California.

E19. The procedures and methods used to identify cows in farm herds. J. D. Burke, Cornell University.

The New Proved Sire Report.

E20. Present status of the new proved sire report. J. F. Kendrick, Dairy Cattle Research Branch, USDA, Washington, D. C.

E21. Usefulness and limitations of the new proved sire and stable-mate record. R. W. Touchberry, University of Illinois.

ANNUAL REPORT OF THE JOURNAL MANAGEMENT COMMITTEE

J. T. Reid
Chairman, Journal Management Committee, Department of Animal Husbandry
Cornell University, Ithaca, New York

During 1959-60, the Journal Management Committee established the following policies: (a) to disallow the reproduction by any persons or agencies, of papers published in the Journal, (b) to allow publication in the Journal by nonmembers of A.D.S.A., and (c) to appoint members of the Editorial Board in numbers, and for a period of service, determined by the need.

The Committee approved on an experimental basis the publication of the new section, Interpretive Summaries of Papers, which was proposed by the Special Publications Committee to serve the general membership. Although the content and style of many of the résumés of the first few issues failed to serve the purpose for which they are intended, and authors generally are opposed to the plan, means of improving the résumés have been instituted and the Committee shall continue to examine the degree of their success.

In the interest of uniformity, readability, and economy, certain changes in the style of the Journal were adopted to become effective in Volume 44 (1961). These concern the size and kind of print, a suggested plan for the uniform arrangement of materials in the Journal, and an arrangement of the Table of Contents intended to focus attention especially on items of general interest.

Other activities included the appointment of George M. Ward, Kansas State University, to the Editorial Board; the publication as a supplement to the May, 1960, issue, of the papers presented at the Fourth Biennial Symposium on Animal Reproduction entitled *Effect of Germ Cell Damage on Animal Reproduction*; and the clarification of the account of the Journal Management Committee's duties recorded in the Administrative Handbook.

PROPOSED AMENDMENTS TO AMERICAN DAIRY SCIENCE ASSOCIATION BY-LAWS

H. F. Judkins, Secretary-Treasurer, White Plains, New York

At the 55th Annual Meeting, the Executive Board authorized a committee to study needed revisions of the Constitution and By-Laws. The Committee, composed of R. E. Hodgson, E. O. Herreid, H. F. Judkins, E. C. Scheidenhelm, and E. L. Jack as Chairman, propose the following changes in the By-Laws. These changes have been approved by the Executive Board and will be voted upon by the membership at the 56th Annual Meeting.

By-Laws

Article I, Section 5 (k) now states:

The Executive Board shall have authority to define and establish awards and to grant honorary and life memberships according to conditions described in Article III of the By-Laws.

Proposed:

The Executive Board shall have the authority to define and establish awards and to grant life memberships according to conditions described in Article III of the By-Laws.

Reason:

Deletes the word, honorary, since the previous honorary membership is now called the Award of Honor and the authority for this is covered under Awards.

Article II, Section 5 now states as Section 4:

The official ballot, containing the nominations of the Nominating Committee and pertinent biographical information regarding each candidate, shall be mailed by the Secretary-Treasurer to each member of the Association on or before May 1. Ballots shall be returned to the office of the Secretary-Treasurer by the members voting.

Proposed:

The official ballot, containing the nominations of the Nominating Committee and pertinent biographical information regarding each candidate, shall be mailed by the Secretary-Treasurer to each member of the Association on or before May 1. Ballots shall be returned within 30 days of mailing to the office of the Secretary-Treasurer by the members voting.

Reason:

This establishes a desirable time-limit for voting.

Article III, Section 3 now reads:

The American Dairy Science Association may honor chosen members for distinguished services or achievements. The Executive Board shall be charged with the responsibility of defining and establishing, or causing to be

defined and established, suitable awards or recognitions to accomplish this purpose. The Executive Board may accept the cooperation, financial or otherwise, of organizations or individuals who may wish to participate in honoring the man chosen to receive such awards or recognitions.

Proposed:

The American Dairy Science Association may honor chosen individuals for distinguished services or achievements. The Executive Board shall be charged with the responsibility of defining and establishing, or causing to be defined and established, suitable awards or recognitions to accomplish this purpose. The Executive Board may accept the cooperation, financial or otherwise, of organizations or individuals who may wish to participate in honoring the individuals chosen to receive such awards or recognitions.

Reason:

Makes it possible to cover the Distinguished Service Award which has been approved by the Executive Board, by changing the word, members, to individuals.

Article IV, Section 1 (a) now states:

Divisions of the Association, organized by members of the Association on the basis of geographical location, may be authorized by the Executive Board upon petition by members of the Association.

Proposed:

Divisions of the Association, organized by members of the Association on the basis of geographical location, may be authorized by the Executive Board upon petition of not less than 25 members of the Association.

Reason:

Establishes a reasonable numerical basis for authorization of the division.

Article IV, Section 1 (d) now states:

Divisions may collect funds and/or dues to be expended for their own purposes.

Proposed:

Divisions may collect funds and/or dues, and in addition, the Association shall grant a subsidy of $25 per annum, unless this sum is modified by the Executive Board. These monies may be expended by the divisions for their own purposes.

Reason:

This alters the By-Laws to conform to authorized practice with respect to the Association subsidy.

Article IV, Section 2 (a) now reads:

Professional groups organized by members within the Association on the basis of specialized interests and to be known as sections, may be authorized by the Executive Board upon petition of not less than ten members.

Proposed:

Professional groups organized by members within the Association on the basis of specialized interests and to be known as sections, may be authorized by the Executive Board upon petition of not less than twenty-five members.

Reason:

Brings the numerical basis for section petitions in line with division petitions.

BOOK REVIEW

PIONEER MICROBIOLOGISTS OF AMERICA. Paul F. Clark, Madison, Wis. University of Wisconsin Press, 1961, pp. 369, $6.00.

In this volume Dr. Clark has chronicled the development of microbiology in America from the time of its earlier beginnings in Europe, through its importation to this country in the last quarter of the 19th Century, and during its growth in the late Victorian and pre–World War I era. This presentation of the history of early American microbiology and its pioneers is largely a personal story of men and achievements, told in a series of short personality sketches. The author's comprehension of the events, his personal acquaintance with many of the personalities, and his ability to fashion a well-turned phrase make it a story well-told.

It will come as no surprise to those familiar with the early development of microbiology that the book deals primarily with the medical and public health aspects of the science. The subject matter has been considered in five major divisions. A four-chapter section on the Foundations of Early Bacteriology introduces the study. Thereafter, the United States is divided into three geographical sections, The Atlantic Seaboard (8 chapters), The Central Valley (2 chapters), and Our Western Lands (2 chapters), for detailed discussion of men, institutions, and achievements. The final section is entitled Perspective and contains two very interesting chapters. The first, Our Means of Communication and Our Early Leaders, summarizes the founding of some of our learned professional societies and journals. The last chapter, Epilogue—From a Laboratory Window, is recommended reading for all who are engaged in the study of the life sciences.

In a critical vein, the reader may feel that his own particular branch of microbiology and/or his institution has not been accorded its proper and rightful number of lines of print. However, the author has forewarned the reader of this possibility, just as he has forewarned that it is a somewhat rambling chronicle.

The author is Emeritus Professor of Medical Microbiology, University of Wisconsin, and former president of the Society of American Bacteriologists. His book, dedicated to the memory of Barnett Cohen, first archivist of the Society of American Bacteriologists and first editor of Bacteriological Reviews, is a well written and interesting summary of early microbiology in the United States. The book, liberally sprinkled with photographs of the subject personalities, quotations from the classics, and references to source material, will be of interest to students and practitioners of microbiology.

W. O. NELSON
University of Illinois, Urbana

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